CHEMO-IMMUNOLOGICAL STUDIES ON LOCALIZED INFECTIONS.

Fourth Paper: Experimental Pneumococcic Meningitis and Its Specific Treatment.*

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The purposes of this paper are to record the general progress of the chemo-immunological studies, and in particular to show that a fatal experimental disease similar to a not uncommon and grave disease of man has often terminated in recovery after a treatment formulated from these studies, and to indicate a way in which the corresponding disease of man may be treated with a reasonable hope of sometimes preventing its almost invariably fatal termination.

In the first paper[1] has been related why and how we were led by consideration and experiment to attempt to arrest the progress of experimental pneumococcic infections by the local use of sodium oleate combined with immune antipneumococcic serum and boric acid. Among others, certain experiments were reported illustrating the results obtained by treating rats and mice inoculated in the peritoneal cavity. It was shown that appropriate treatment early after inoculation with massive doses of virulent pneumococci not only regularly saved the animals but also prevented the appearance of more than the slightest transitory signs of any illness. As a measure for preventing the development of infection the treatment was effective. As a curative measure for arresting the developing, or already established infection it was less effective, particularly in respect to the lapse of time after inoculation. Yet if we consider the severity of the test, the animals being highly susceptible to

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[1] Lamar, R. V., *Jour. Exper. Med.*, 1911, xiii, 1.

rapidly fatal septicemia, the results were encouraging enough to lead us to pursue the principle in the hope of developing it, and because of the promise which it afforded of a possible successful application to the treatment of certain localized pneumococcic infections of man.

Since the publication of the first paper the work has been continued along the same fundamental lines in the search for an improved means of treatment. At the same time, pending developments in this direction, we undertook to ascertain whether what was already in hand might be successfully applied to the treatment of an experimental disease which bears a closer resemblance to pneumococcic diseases of man than is afforded by the septicemic infections of the small laboratory animals, and thus offers more reliable indications of the practical value of the therapeutic principle.

In the second and third papers[2] we recorded the results of the search for a more efficient agent than sodium oleate. Several substances were found to be much more powerful in destroying the pneumococcus in the test-tube, but they were less efficient than sodium oleate in controlling infections of the animal body. Also the attempts to find an agent which is superior to boric acid in preventing the serum inhibition of the sodium oleate failed. Hence we turned to the determination of the optimum proportions of the components of the therapeutic mixture of sodium oleate, immune serum, and boric acid, which had already proved itself.

What is meant by the optimum composition of the mixture will be apparent upon a brief restatement of the essential points regarding the action of the components alone and combined. The destructive action upon the pneumococcus of sodium oleate solutions varies throughout a wide range of concentration in direct ratio to the degree of dissociation. The destructive action is prevented wholly or in part by blood serum according to quantitative conditions. Alone in the animal body sodium oleate has little or no apparent restraining influence upon pneumococcic infections. In the test-tube a potent immune serum kills many of the pneumococci present but allows a subsequent rich growth of those which escape destruction, unless indeed a very small number be present, when all may be killed. The bactericidal action of immune serum does not depend

[2] Lamar, R. V., *Jour. Exper. Med.*, 1911, xiii, 380; xiv, 256.

wholly upon, but is enhanced by, the presence and coöperation of phagocytes; and the intensity of action varies in direct ratio to the degree of concentration of the serum. In the animal body immune serum exerts a distinct protective action, but one efficient in saving the animal only under quite sharply defined conditions pertaining to quantity of culture and of serum. In other words, there is a minimum quantity of serum which will protect against even the most minute, otherwise certainly fatal quantities of culture, and no quantity of serum protects against more than a definite quantity of culture. Dochez[3] directed attention to this phase of the protective action of immune serum and has recently made it the subject of a special investigation. He has pointed out the relationship between quantity of serum and of culture in determining protection or lack of it, and suggested the importance of its bearing upon the practical treatment of established infections with immune serum. We attach an additional importance to the facts as affording a standard of comparison for appreciating the greater efficiency of the mixture of sodium oleate, immune serum, and boric acid. In contradistinction to test-tube experiments, a given quantity of serum in the animal body is just as efficient, if previously diluted even to 10 per cent. with salt solution, as if employed undiluted. The presence of boric acid in a certain quantity is necessary to prevent serum inhibition of the oleate, but an excess defeats the purpose of its employment, since of itself, in large quantities, it prevents the bacteriolytic action of the sodium oleate.

After testing several species of animals in diverse portions of the body in which we attempted to produce a suitable disease, with strains of the pneumococcus varying in virulence, we finally employed for our study pneumococcic meningitis in monkeys produced by a highly virulent pneumococcus. The conditions which this experimental disease presents are favorable for testing the method and important because of their close similarity to the highly fatal pneumococcic meningitis in man.

The experimental disease could be produced with ease, and, as it appeared later, with certainty. Except necessarily in the mode of infection it was remarkably like pneumococcic meningitis of man.

[3] Dochez, A. R., Personal communication.

The clinical, bacteriological (including bacteremia), and pathological manifestations were nearly identical. It differed in that its course was more severe, and when untreated it terminated invariably in death. This statement is true of the disease produced, as it always was except once, by virulent pneumococcus. In a single instance a less virulent pneumococcus was injected and a meningitis produced that terminated in recovery without any intervention other than lumbar puncture performed for diagnostic purposes. The experiment has no bearing upon the particular theme of the paper, but is recorded to show how much the clinical result depends upon the infecting bacterium. It is an experimental confirmation of what clinicians are coming to realize, namely, that pneumococcic meningitis though a highly mortal disease is not necessarily always fatal; and it serves to indicate still further the close similarity of the experimental to the spontaneous disease.

The disease lent itself well, moreover, to constant observation of its course, and to direct local treatment, as lumbar puncture afforded indications of the influence of treatment during the course of the disease.

Before recording the individual experiments certain general features should be considered relating to the animal infected, the kind of pneumococcus employed, the mode of infection, and the method of treatment.

Macacus rhesus was chiefly used, but a few experiments were made with *Cercopithecus callitrichus.* All the monkeys were vigorous and seemed in good health. Genus, species, and sex appeared not to influence the results. But the individual factor was manifest. Most animals reacted to the inoculation in such a manner as to permit ready recognition of the type of the disease. Occasionally an individual proved excessively susceptible, seemed to oppose no resistance, and died of septicemia within the day. The monkeys seemed to be less resistant in the cold than in the warmer months. When and in what way these two factors of the individual and the season appeared to influence the experiment will be indicated in the discussion of the protocols.

The first three experiments were made with different strains of pneumococcus. They showed that a meningitis could be readily

produced, and allowed a study of the characteristics of the disease and the influence of degree of virulence of the bacterium upon its course. From the third experiment on, the same strain of pneumococcus was used throughout. This organism was obtained in pure culture by Dr. Dochez directly from the circulating blood of a patient with lobar pneumonia. Suffice it to say that it was a characteristic pneumococcus soluble in bile. In the beginning the organism was of high but not extraordinary virulence. It was first inoculated into monkeys after five passages in mice when 0.000,001 of a cubic centimeter was fatal. Repeated control tests showed the degree of virulence to be maintained throughout the ten months of study. In the nature of its pathogenic action the organism was well adapted to the purposes: it caused inflammation rather than a rapidly fatal septicemia.

In the first experiment the culture used was a virulent pneumococcus grown from the spinal fluid of a patient with meningitis. In the second one a so called avirulent pneumococcus isolated directly from the saliva of a well person was employed. The characteristics of this organism are stated in the protocol of monkey 2.

At the beginning a few experiments were made by intracranial inoculation. The disease produced in this way was most severe and quickly caused the death of the animal. Besides, it did not lend itself well to the treatment which is preëminently a local one administered by lumbar puncture. The opportunities for fluid injected intraspinally to reach the distant seat of severe inflammation in the brain itself were small. Normally in the monkey the space in the membranes is small and this is quickly further reduced by swelling and the fibrinous exudate. Indeed the focus of infection in the brain and its membranes seemed sometimes to have been cut off from the spinal subdural space. On account of the fibrinous nature of the pneumococcic inflammation this meningitic disease offers greater difficulties in treatment than, for instance, epidemic cerebrospinal meningitis. We shall refer later to those cases of pneumococcic meningitis which arise from middle ear disease and where the cerebral meninges are first and most inflamed.

Hence the intracranial method of infecting the animal was abandoned early, and throughout the remainder of the work the animals

were inoculated and treated by subdural spinal puncture and injection.

Two kinds of immune serum which were efficient in protecting rats and mice against infection by the same strain of pneumococcus used to produce the meningitis were tested alone. The one was from a goat, the other from a horse, immunized by the intravenous injection of large quantities of live, highly virulent, homologous pneumococci. Later other monkeys were treated with a mixture of sodium oleate, the same immune serum, and boric acid.

The experiments are presented in three groups. Those in the first group show the nature of the untreated disease and at the same time serve as control observations from which the results of treatment may be deduced. Those in the second group show the results of treatment with immune serum; and those in the third, treatment with the mixture of sodium oleate, immune serum, and boric acid.

THE EXPERIMENTAL DISEASE.

The first experiment was performed by Dr. Flexner in the spring of 1910. We abstract from his notes:

May 24, 1910. Monkey 1. *Macacus rhesus;* medium size. 2:00 P.M. Lumbar puncture; clear fluid; injected 0.4 c.c. of a thin suspension of a 24 hour culture on sheep serum agar, and a few drops of spinal exudate, from a case of pneumococcic meningitis (Schirck). 7:00 P.M. On perch; hair erected; not very sick. May 25, 10:00 A.M. Not very sick. L. p.;[4] small quantity of clear fluid containing a few polymorphonuclear leucocytes; no cocci. Injected 0.5 c.c. of a bouillon suspension from a fresh culture of same organism. 2:00 P.M. Monkey sick, lying partly down on perch. 4:00 P.M. L. p.; no fluid runs from needle. A little pus mixed with water remaining in needle is drawn up by the syringe. Film shows polymorphonuclear leucocytes and some Gram positive diplococci intra- and extracellular. May 26, 9:00 A.M. Animal lying on side; very sick. 10:00 A.M. General convulsion. 11:00 A.M. L. p. Cloudy fluid containing flakes of cells. Many leucocytes and diplococci some of which are intracellular. 11:10 A.M. General convulsion; some retraction of head; nystagmus; body limp.

May 27, died at 9:30 A.M. Autopsy at 11:00 A.M. The vessels of the cerebral meninges are injected and there is cloudy fluid in the meshes of the pia arachnoid, especially over the medulla. The ventricles contain an excess of cloudy fluid, but no purulent exudation. No macroscopic changes in the other organs. Films from the cerebral meninges and choroid plexus show many

[4] Abbreviation for lumbar puncture.

diplococci, nearly all extracellular. Many diplococci in heart's blood and spleen. Pure cultures of pneumococcus from surface of brain, lateral ventricle, heart's blood, and spleen.

The experiment requires little comment. Clinically there were many of those manifestations of meningitis which occur in man. Septicemia supervened. Death occurred early. At autopsy the familiar morphological and bacteriological conditions were encountered.

In the second experiment an organism of that variety which is commonly called avirulent was used. It was isolated directly from the saliva of a well person on January 25, 1911. The colony was of the characteristic ring form. In subcultures the organism exhibited the typical cultural and morphological characters of the pneumococcus; it fermented inulin feebly, and was soluble in bile. 0.5 of a cubic centimeter of a broth culture proved harmless to a mouse. From the time of its isolation until the date of this experiment, i. e., for ten weeks, the organism had been kept continuously on plain agar at 37° C. by third day transplantations.

Apr. 4, 1911. Monkey 2. *Cercopithecus callitrichus;* rather large. 3:30 P.M. Under ether anesthesia injected into cranium through fine trephine opening in left frontal region 1 c.c. of a 24 hour plain broth culture of pneumococcus L. 9. Animal conscious in five minutes. 4:30 P.M. Bright and active just as before inoculation. Apr. 5, 9:30 A.M. Animal sits on perch with head bowed; hair over anterior part of body erected. No paralysis or other focal symptoms. 3:30 P.M. A little worse; entire coat erect; little resistance to being captured. L. p. 1 c.c. of distinctly turbid fluid drops from needle; no coagula. Many pus cells and typical diplococci mainly extracellular; very little phagocytosis. Transplantation of one loop of fluid affords many colonies of pure pneumococcus. Apr. 6, 9:00 A.M. Animal lively; does not appear ill; coat smooth. 10:00 A.M. L. p. 4 drops of turbid fluid slowly. Many p. n. l.,[5] but fewer than yesterday; moderate number of large, and a few small, mononuclear leucocytes. Fewer diplococci than in yesterday's fluid; no phagocytosis. Slight pure growth of pneumococcus. Apr. 7. Animal seems well. Apr. 10. Well. May 8. Continued well.

The experiment has already been referred to in the introduction. It led to the trial of another strain of pneumococcus, and one known to be virulent. The value of lumbar puncture in affording important information is apparent.

[5] Abbreviation for polymorphonuclear leucocytes.

In all the experiments now to be reported the virulent culture described before was used.

Apr. 9, 1910. Monkey 3. *Cercopithecus callitrichus;* very large. 11:30 A.M. Under ether anesthesia injected into cranium 1 c.c. of a 24 hour plain broth culture of pneumococcus $X.5^{2}$.[6] Animal is recovering while the wound is being closed. Recovery complete in a few minutes. Apr. 10. Animal found dead at 7:00 A.M. Death occurred about 19 hours after inoculation.

9:30 A.M. *Autopsy.*—Upper lip retracted; both eyelids swollen; pupils one half dilated, round, equal. Lumbar puncture; two drops of slightly turbid fluid; very few p. n. l.; myriad diplococci; many typical pairs, also short chains; many degenerative forms. The brain shows yellow through the dura mater. The pia is milky and covered everywhere with a viscid exudate, greatest at the base of the cerebrum. The lateral ventricles contain a small quantity of the same exudate. The pia of the cord is milky everywhere, and there is a scant exudate. The ventral cavity of the body presents no gross changes. Films from the surface of the cerebrum, the lateral ventricle, and the spinal cord disclose many p. n. l. and a great many cocci in pairs and short chains, with only an occasional example of phagocytosis. In the heart's blood are many typical pairs and short chains. The spinal fluid, lateral ventricle, and the heart's blood all afford a pure profuse growth of pneumococcus.

The quantity of culture was large, and the disease fulminant, septicemia quickly supervening. The experiment indicates how quickly the spinal meninges may become involved and how rich the early exudate may be in fibrin. The bearing of the nature of the exudate upon the efficacy of treatment is most important, as will appear in the succeeding protocols.

The next experiment was performed in the same way with a smaller quantity of culture.

Apr. 10, 1911. Monkey 4. *Cercopithecus callitrichus;* medium size. 11:40 A.M. Ether anesthesia; injected intracranially 0.1 c.c. (1 c.c. of a 1 to 10 broth dilution) of a 24 hour broth culture of pneumococcus $X.5^{3}$. Complete recovery in a few minutes. 5:00 P.M. Animal seems well. Apr. 11, 9:15 A.M. Animal sits bent forward slightly; coat slightly raised; eyes watery; pupils half dilated, round, and equal; respiration moderately accelerated. L. p. 3 drops of slightly turbid fluid very slowly; very few p. n. l.; few typical pairs of extracellular pneumococci. Moderate pure growth from one loop of fluid. P.M. Animal perhaps slightly worse. Apr. 12, 9:30 A.M. Worse; reclines on floor of cage in the corner; coat erect. Several attempts to obtain spinal fluid are unsuccessful. In the afternoon the animal's condition grows worse. Apr. 13,

[6] The Roman numeral refers to the name of the culture; the Arabic decimal indicates the serial number of the last mouse "passage"; and the exponent the generation in broth used.

A.M. Much weaker; lies on floor with head retracted. Made two unsuccessful attempts to obtain spinal fluid. During the afternoon the weakness slowly progresses.

Apr. 14. Found dead at 7:50 A.M. The body is fresh. Death occurred about 3½ days after inoculation. Autopsy 12 M. The inflammation is intense over the vertex of the cerebrum, and gradually diminishes towards the base and the medulla to become slight throughout the meninges of the cord. The cerebral cortex is yellow and the sulci are distended with pus and fibrin. The ventricles contain a moderate quantity of turbid fluid. Films from the brain show many p. n. l. and very many cocci in pairs and short chains; fewer in the scant spinal exudate. Profuse cultures from cortex, lateral ventricle, spinal cord, and heart's blood.

With the smaller quantity of injected culture the disease was slower in developing and the local inflammatory reaction was more marked. Spinal fluid could not be obtained after the end of the second day probably because of the thickening of the meninges and the absorption of the fluid by the fibrin in the exudate. The usual richness of the exudate in fibrin, a characteristic of pneumococcic inflammations in general, has in this particular disease an indirect effect in materially lessening the quantity of fluid which may be injected and in hindering its diffusion throughout the spinal canal and into the cranium.

The remainder of the experiments grouped under this heading were performed at intervals during the work largely for the purpose of often controlling the virulence of the culture, and the effect of treatment in companion experiments. In each of them the culture was injected by the spinal route.

May 8, 1911. Monkey 17. *Macacus rhesus;* medium size.

9:45 A.M. Injected, intraspinally, after the free withdrawal of 1.5 c.c. of clear fluid, 0.1 c.c. (1 c.c. of a 1 to 10 broth dilution) of a 24 hour broth culture of pneumococcus $X.5^{11}$. No symptoms. May 9, A.M. No signs of illness. 2:45 P.M. Animal is restless and eats less than usual. L. p. 1.2 c.c. of slightly turbid fluid freely. Sediment obtained by centrifugalization; many p. n. l.; few large and small mononuclear leucocytes; few extracellular diplococci. Profuse growth in culture. May 10, 9:00 A.M. Animal sits bowed on perch; hair raised slightly; weakness in movements upon being disturbed. 10:45 A.M. L. p. 12 c.c. less turbid fluid freely; several small white flakes; fewer cells; many cocci in pairs and a few short chains; slight phagocytosis. Profuse growth in culture. P.M. Animal becomes worse with great rapidity and dies at 5:00 P.M., 2¼ days after inoculation.

Autopsy.—May 11, 9:00 P.M. The spinal membranes are moderately congested, moist, and the pia is milky. No fibrin is visible. The cerebral meninges

are glued together, and the cortex tears in several places as the dura is being reflected. There is a general moderate congestion most marked at the base. The pia is cloudy everywhere and opaque in the sulci. The ventricles contain a small quantity of rather thick yellowish fluid. In films from the cortex there are a great many pus cells and many diplococci for the most part extracellular and only fairly well preserved. There is moderate phagocytosis. In the spinal exudate there are fewer pus cells and diplococci and less phagocytosis. On the surfaces of the lower lobes of both lungs is much mucilaginous exudate in sheets and clumps containing many poorly staining pus cells and a moderate number of typical pneumococci, of which a few are intracellular. Otherwise the ventral cavity is negative. No bacteria seen in the heart's blood; culture negative. From the cerebral and spinal exudate the pneumococcus grows profusely and pure.

At first the animal offered considerable resistance, shown by its physical condition, the polymorphonuclear cell reaction in the spinal fluid, and the slow multiplication of the diplococcus. Then it showed signs of illness and became worse with great rapidity, while coincidently the cellular reaction subsided and the cocci grew profusely. The absence of cocci from the blood at autopsy indicates that the meningitis itself may cause death without the supervention and help of septicemia. There was a metastatic pleurisy of slight extent and degree.

The experiment reported next was done in the autumn after the summer interruption of two months, during which time the culture was preserved in the dried spleens of infected mice.

Sept. 19, 1911. Monkey 32. *Macacus rhesus;* medium size.

11:00 A.M. L. p.: three attempts to obtain fluid failed; on the third attempt. injected 0.1 c.c. (1 c.c. of a 1 to 10 broth dilution) of a 24 hour broth culture of X.9[1]. With the needle and syringe still in place withdrew and reinjected 0.3 c.c. for assurance. No symptoms. During remainder of the day the animal seems well. Sept. 20, 9:00 A.M. Animal not so active as yesterday. 11:00 A.M. Ill; rather quiet; deliberate in movements; eyes dull; coat beginning to rise. 2:00 P.M. All symptoms more marked; weakness evident as animal springs; head retracted at times. 4:00 P.M. Worse; coat much raised. Sept. 21, A.M. Animal is worse; quite weak, but still moves about; coat rough. P.M. Condition about the same. Sept. 22, 9:22 A.M. Animal much worse; no longer jumps but climbs slowly up grating of cage to perch. P.M. Very weak; sits quietly huddled; does not move when touched. Sept. 23, 9:00 A.M. Prone; apparently dying; head much retracted. Death at 10:30 A.M., 4 days after inoculation.

Autopsy.—Performed at once. The brain and cord are moist. The pia everywhere is thickened, opaque, and yellowish white, more over the cerebral hemispheres than along the cord. The ventricles are slightly distended with turbid

fluid. Diplococci are present all along the cord and over the brain beneath the dura. They are not numerous except in the fibrinous exudate covering and infiltrating the pia of the convexity of the brain, where there are enormous numbers. Phagocytosis is slight. At the bases of both lungs there are a few deposits of a dry fibrinous exudate containing pus cells and diplococci. From the cortex, lateral ventricle, spinal cord, and pleural exudate the pneumococcus grows profusely; from the heart's blood, in a few isolated colonies.

The experiment indicated that the organism had preserved its virulence. The disease progressed gradually and steadily. Again the inflammation appeared to be greater in the cerebral than in the spinal meninges although the inoculation was made into the spinal canal. The dry pleurisy occurred again. And, as in the preceding experiment, septicemia did not seem to be necessary to cause death. The few cocci found in the blood at autopsy were probably the expression of an agonal invasion rather than of a true septicemia. The animal of this experiment was the companion to one inoculated at the same time in the same way and treated later (compare monkey 33 of the third group).

Dec. 12, 1911. Monkey 45. *Macacus rhesus;* medium size. 1:55 P.M. L. p.: about 0.7 c.c. of clear fluid; injected 0.1 c.c. of a 22 hour broth culture of X.11[5]. Slight retraction of back upon completion of injection passing away in a few seconds. For the remainder of the day until 6 o'clock, when it is observed for the last time, the animal seems perfectly well. Dec. 13. Animal found dead at 8:00 A.M. Lived about 15 or 16 hours after inoculation.

Autopsy.—At 9:30 A.M. Body fresh. There is a moderate quantity of yellow, sticky exudate all along the spinal pia, and over the brain, being most abundant at the base. The ventricles contain a modicum of opalescent fluid. In the meningeal exudate everywhere there are many pus cells and enormous numbers of diplococci, many of which are intracellular. Cultures are profuse and pure. Films from the left lateral ventricle show a few mononuclear cells, no p. n. l., and no cocci. No growth upon transplantation from this source. Heart's blood: moderate number of typical capsulated pairs and a few short chains; profuse growth in culture. Both lungs are slightly congested and edematous, but there is no consolidation. Otherwise the ventral cavity is negative.

This experiment shows the fulminant disease. It is remarkable that so intense a purulent inflammation with fibrin formation could occur in such a short time. At this season, during the cold of midwinter, the disease ran regularly a shorter course than it had done in the spring and autumn. Possibly also this animal was unduly susceptible, for no change was ever made in the manner of preserv-

ing and of using the culture, which in all other tests exhibited what is commonly accepted as maximum virulence.

Two more experiments to illustrate the untreated disease very similar to the one just described may be summarized. The monkeys were inoculated in the usual way late in the afternoon of December 14, 1911, and on January 5, 1912, respectively. Seen twenty and forty-five minutes later the animals seemed well; but both died during the night, one about thirteen hours, and the other about nineteen hours after the inoculation. The autopsy in each instance showed a general purulent cerebrospinal meningitis and septicemia. The rapidity of the development is evident.

It was, as already stated, in mid-winter that the disease was so much more severe than it had been before. Therefore the quantity of culture inoculated was reduced to one fifth of what had been regularly used before. The protocols of the experiments appear in the second and third groups.

To summarize: the experiments show that virulent pneumococci when injected into the posterior cavity of monkeys produce regularly an inflammation of the meninges, and particularly of the pia mater, attended by bacteremia; that the inflammation becomes quickly purulent and fibrinous in character, extends readily from the cerebral to the spinal meninges and *vice versa,* and is so regularly attended by certain definite general symptoms that the whole constitutes a definite disease entity.

INFLUENCE OF TREATMENT WITH IMMUNE SERUM.

In all, eleven experiments were made with immune serum. In two not only the animal was treated but also the culture before it was inoculated. That is, in order to make the simplest test of the serum, a large quantity, many times that required to protect a rat against the same quantity of culture as the monkeys received, was added to the culture, the mixture incubated, and then inoculated into the monkey, which later received still more serum. In nine experiments of this group, as well as in all those of the third group, the animal was first inoculated with the culture alone and then later treated. The time allowed to elapse between inoculation and

the beginning of treatment was usually determined by the physical condition of the animal and the character of the spinal fluid. As a rule, in the treatment with immune serum alone, this interval was short. The results of early treatment were so little encouraging that there was no reason to increase the interval between inoculation and the beginning of serum treatment. Once begun the treatment was usually repeated once a day as long as the animal lived; or, as occurred in two instances, until the physical condition of the animal was much improved and the spinal fluid gave no growth of the coccus. The quantity of serum injected was the maximum of what was considered safe, and was determined by the quantity of fluid which had been withdrawn, and the condition of the animal while the slow injection was being made. In the beginning of the disease it was almost always possible to inject two cubic centimeters with no apparent embarrassment to the animal; later, as the disease progressed, less spinal fluid could be withdrawn and less serum injected, but even then it was usually possible to give at least one cubic centimeter without appreciable harm. In some experiments concentrated serum was used; in others a 40 per cent. dilution. Of itself the serum seemed to be harmless.

Apr. 14, 1911. Monkey 9. *Cercopithecus callitrichus;* large size.

9:55 A.M. Under ether anesthesia injected intracranially 0.1 c.c. of a 24 hour broth culture of X.5[4]. Recovery complete in a few minutes. 1:55 P.M. No signs of illness noticed. L. p.: 0.5 c.c. of slightly blood-tinged fluid. In the sediment there are no more leucocytes than the presence of blood accounts for, and no cocci are seen. Twelve colonies of pneumococcus grow from the planted fluid.

Injected 2 c.c. of immune serum (goat 5, bleeding of February 16, 1911). No symptoms attend or follow injection.

Apr. 15, A.M. Animal is slightly ill; sits quietly but moves with agility when disturbed; coat somewhat fluffy. 10:15 A.M. L. p.: 2 drops of clear fluid; very few cells, about half of which are polymorphonuclear and half mononuclear; no cocci seen. Scant growth of pneumococcus in culture. Injected 2 c.c. of the same immune serum.

Apr. 16, 10:15 A.M. Animal slightly worse; not so quick in movements; head droops a little. L. p.: no fluid obtained. Injected 2 c.c. of immune serum without resistance. Apr. 17, 10:30 A.M. Slightly worse. L. p.: no fluid obtained. Injected 2 c.c. of immune serum; slight struggle towards close of injection. Until noon the animal remains in about the same condition. P.M. Much worse; lies down sometimes; no longer eats.

Apr. 18, 10:00 A.M. Very weak; lies on side, eyes swollen. L. p.; no fluid

obtained; believing that the animal would die if left alone, we injected again 2 c.c. of immune serum. The last half of the injection is attended with slight struggle and embarrassment of respiration. During the remainder of the day the animal's condition seems about the same as before the injection. Apr. 19. Animal found dead at 8:00 A.M. It died $4\frac{5}{6}$ days after inoculation.

Autopsy at 10:00 A.M. Intense inflammation of cranial meninges; the sulci are widely distended by a purulent exudate rich in fibrin. There is a small area of red softening on the superior surface of the right lobe of the cerebellum. The spinal cord is enveloped throughout by a yellowish sheath about 2 mm. thick representing the pia and the fibrinopurulent exudate. There are many p. n. l. and enormous numbers of cocci in pairs and short chains in the cerebral and spinal exudate. Phagocytosis is slight. In culture profuse pure growth of pneumococcus from exudate and heart's blood.

In this experiment treatment was begun early and only four hours after the inoculation while the animal still seemed well; and although the bacteria had already reached the spinal fluid probably only slight multiplication had occurred, for they were too few in number to be seen in films. Even on the second day the animal was still very strong. At the best the serum only delayed the fatal issue a little while. It may not have reached the brain in more than small quantities much diluted, but even in the spinal canal where it was present and could not have been greatly diluted it did not prevent the growth of the cocci and the production of an inflammation almost as severe as that in the cerebral meninges.

Apr. 21, 1911. Monkey 11. *Macacus rhesus;* large.

11:10 A.M. Under ether anesthesia injected intracranially 0.1 c.c. of a 24 hour broth culture of X.5[6]. Complete recovery at once. 3:10 P.M. Animal seems a little torpid; otherwise no signs of illness are detected. L. p.: 1.2 c.c. of turbid fluid freely; moderate number of p. n. l., more than a moderate number of extracellular diplococci. Profuse growth in culture. Injected easily 2 c.c. of immune goat serum. No symptoms. Also injected intravenously 5 c.c. of immune serum. This injection is followed by a mild shock of short duration. Seven minutes later the animal has recovered completely. 4:30 P.M. The animal huddles against another in the cage; moves with agility when disturbed; coat slightly raised. The animal was observed no more this day, and was found dead at 7:30 the next morning. It probably lived 8 or 9 hours after the inoculation.

Autopsy.—11:30 A.M. The membranes of the brain, chiefly over the convexity, are milky from the presence of a moderate quantity of yellowish white exudate containing some fibrin. Films show many p. n. l. and very many diplococci. In the spinal canal there is less exudate but yet p. n. l. and many diplococci. The heart's blood contains many diplococci. Profuse growth of pneumococcus occurs in all cultures.

In this experiment the animal was unusually susceptible, for in four hours it had become ill and the bacteria had reached the spinal fluid and begun to multiply. It was on this account that the intravenous injection of immune serum was made. At least it was not effective in delaying death. The experiments were so little encouraging that an attempt was made to afford the serum better opportunities to exert any beneficial action of which it might be capable. To this end the culture and serum were mixed and incubated and then injected in order to see if infection would be prevented.

Apr. 21, 1911. Monkey 12. *Macacus rhesus;* small.

4:00 P.M. Under ether anesthesia injected intracranially a mixture consisting of 0.1 c.c. of a 24 hour broth culture of X.5[6] and 1 c.c. of immune goat serum. The mixture had been incubated for 1 hour and 10 minutes at 37° C. and then kept in the refrigerator for 30 minutes. A culture of the mixture just before it was injected furnished a moderate growth of pneumococcus. The animal quickly recovered and seemed well for the remainder of the day. Apr. 22. The animal may be a little agitated; otherwise it seems well. 11:00 A.M. L. p.: 1 c.c. of turbid fluid freely; a moderate number of p. n. l., a few mononuclear cells, and no cocci. No growth in culture. Apr. 23. Animal seems well throughout the day.

Apr. 24, A.M. Still seems well. At 3:30 P.M. the animal is first noticed to be ill. It is greatly agitated, shaking at the grating of the cage. In a few minutes it lies prone and trembling with roughened coat. On being taken up an almost complete flaccid paralysis of the left anterior extremity and a spastic paresis of the left posterior extremity is apparent. There is slight ptosis of the left upper eyelid. 4:00 P.M. Animal weaker. L. p.: turbid fluid flows quickly under pressure; a few p. n. l. and mononuclear cells; more than a few diplococci; no phagocytosis; profuse growth in culture. Injected immune goat serum, stopping at 1.6 c.c. upon the appearance of struggle and slight embarrassment of respiration; recovery in one minute. 6:00 P.M. Condition about the same as before the injection. Animal found dead at 7:30 P.M., $3\frac{1}{8}$ days after the inoculation.

Autopsy.—April 25, 10:30 A.M. There is an intense fibrinopurulent inflammation of the pia of the brain and cord, which is more pronounced in the brain where the sulci are widely distended by a thick yellow exudate. The cortex everywhere is much congested, even within its substance. There is a large area of red softening in the right temporal lobe. The ventricles contain a moderate quantity of turbid fluid. In films from the brain and cord there are many p. n. l. and enormous numbers of diplococci everywhere. Profuse growth in cultures from the brain and heart's blood.

In this experiment the immune serum had already effected a considerable reduction in the number of viable cocci in the culture be-

fore it was injected. The condition of the animal for the first two days and the character of the spinal fluid seemed to indicate that the infection if not prevented was retarded. Then symptoms of illness appeared more or less suddenly and the animal very rapidly weakened and died at about the time the untreated controls did. The autopsy indicated that the disease was developing all the while the animal showed few or no chemical signs and explained the focal symptoms.

It was at this point that the intracranial method of inoculating was abandoned and the intraspinal substituted. The next experiment resembled the one just described except that the mixture of culture and serum was injected into the spinal canal, and the animal itself was later given a regular course of serum treatment regardless of its apparent condition.

Apr. 25, 1911. Monkey 13. *Macacus rhesus;* medium size.

1:30 P. M. Injected intraspinally a mixture consisting of 0.1 c.c. of a 24 hour broth culture of X.5[6] and 1 c.c. of immune goat serum. The mixture had been incubated for one hour at 37° C. A transplantation from it just before it was injected furnished a moderate growth of pneumococcus. No symptoms followed the injection. Remainder of the day animal seems well. Apr. 26. Animal seems perfectly well. 10:00 A.M. L. p.: 1.5 c.c. of moderately turbid fluid freely; a moderate number of p. n. l. and a few mononuclear cells are seen in films. There are a few bodies, about half intra- and half extracellular, which appear to be swollen cocci. Here and there an occasional diplococcus is identified. Transplantation gives rise to no growth. Injected 1.8 c.c. of immune serum; considerable struggling. During the afternoon the animal seems well. Apr. 27, 2:50 A.M. No evidence of illness. L. p.: 1 c.c. of fluid, about half as turbid as that obtained yesterday, flows slowly; p. n. l. predominate; no bacteria seen; no growth occurs. Injected 2 c.c. of immune serum; only slight struggling. Apr. 28. Animal seems perfectly well. 9:45 A.M. L. p.: 1 c.c. of faintly turbid fluid; a few cells about half of which are mononuclear; no bacteria seen; no growth occurs. Injected 1.8 c.c. of immune serum; much struggling. Apr. 29. Well.

The animal remained well for one month when it developed diarrhea, became emaciated rapidly, and died on June 30.

Autopsy at 4:00 P.M. Body much emaciated. Nothing abnormal is discovered in the central nervous system. There is an extensive ulcerative enteritis and hyperplasia of the mesenteric lymph nodes. Transplantations from the cerebral cortex, lateral ventricle, spinal cord, and heart's blood give rise to no growth.

In this experiment the immune serum was given the maximal advantage. When incubated with the culture before inoculation

and later administered three times to the animal it prevented the occurrence of infection. Such prevention of infection, while undoubtedly indicative of a beneficial action of the serum, is to be sharply distinguished from the arrest of an established infection. In the next experiment the test of the serum was made somewhat more difficult.

May 1, 1911. Monkey 16. *Macacus rhesus;* medium size.

11:50 A.M. Injected intraspinally 0.1 c.c. of a 24 hour broth culture of X.5°. At 1:50 P.M. animal well. L. p.: 3 drops of opalescent fluid slowly; a few mononuclear cells and an occasional pair of cocci in films; slight growth in culture. Injected 2 c.c. of immune goat serum. Animal seems well remainder of the day. May 2, 9:40 A.M. Animal may be a little agitated; otherwise no signs of illness can be detected. L. p.: about 0.7 c.c. of a slightly turbid fluid containing one small white flake; a fair number of white cells, half p. n. l. and half mononuclear; no bacteria seen; no growth. Injected 2 c.c. of immune serum. May 3, A.M. The animal eats with normal greed and seems well. 11:10 A.M. L. p.: 1 c.c. of opalescent fluid slowly; more white cells than yesterday; ratio about the same; no bacteria seen; no growth. Injected 2 c.c. of immune serum. May 4, 10:05 A.M. Animal has continued well. L. p.: 2 drops of clear fluid; an occasional p. n. l. and a very few mononuclear cells; no bacteria seen; no growth. No serum injected.

The animal was kept under observation for two months during which time it remained well.

Immune goat serum given two hours after the inoculation and once on each of the two succeeding days seemed to prevent multiplication of the bacteria and the occurrence of infection, although the animal was apparently intoxicated for one day after the inoculation.

These two were the only instances in which an immune serum prevented the infection and death of the animal. In all other experiments where a longer period than two hours was allowed to elapse between inoculation and the beginning of treatment not only did all of the animals die, but the disease followed much the same course as in the untreated control animals. These experiments are summarized in table I (page 598).

The experiments call for little discussion. They show that immune serum had a distinct, though only slight, restraining influence upon infection. When administered within two hours it prevented the occurrence of infection in two instances. When given

TABLE I.

Date.	Designation of animal.	Time elapsing between inoculation and beginning of treatment.	At beginning of treatment.		Subsequent treatments.	Result.	Summary.
			Condition of animal.	Nature of spinal fluid.			
May 10, 1911	No. 19[7]	8 hrs.	Slightly ill, excited.	Very few p.n.l., many extracellular diplococci; profuse growth.	None.	Died 20 hrs. after inoculation.	Septicemia; animal was probably less resistant than the average.
May 12, 1911	No. 21[7]	4 hrs.	Slightly ill.	Slight multiplication of cocci; slight growth.	1 and 2 days later.	Died $2\frac{5}{6}$ days after inoculation.	Serum seemed to exert some restraint on growth of cocci; no septicemia.
Oct. 10, 1911	No. 36[8]	18 hrs.	Apparently well.	Few p.n.l.; myriad diplococci; profuse growth.	1 and 2 days later.	Died $3\frac{1}{6}$ days after inoculation.	Bacteremia throughout illness; encephalitis; cerebral softening. Septicemia.
Jan. 11, 1912	No. 50[8]	10 hrs.	Perhaps a little excited; otherwise well.	Very few diplococci, some intracellular; scant growth.	1 day later.	Died $5\frac{1}{6}$ days after inoculation.	The 2d lumbar puncture revealed no cocci either in films or culture, and the animal seemed well on the 2d, 3d, and 4th days. On the morning of the 5th day, the animal showed signs of "relapse," weakened very rapidly, and died a few hours later.
Jan. 24, 1912	No. 52[8]	$8\frac{1}{6}$ hrs.	No signs of illness.	Many p.n.l. and extracellular diplococci; profuse growth.	1 and 2 days later.	Died $2\frac{1}{6}$ days after inoculation.	The first injection seemed to restrain growth of the cocci, the second and third did not do so appreciably.
Jan. 30, 1912	No. 53[8]	$8\frac{1}{6}$ hrs.	Slightly ill; restless.	Many p.n.l. and extracellular diplococci; profuse growth.	None.	Died 21 hrs. after inoculation.	Inflammation mainly cerebral; no spinal fluid at autopsy. Many diplococci in cerebral exudate. No septicemia.

[7] Immune goat serum was employed.

[8] Immune horse serum was employed.

later the first injection seemed usually to restrain infection, but the restraining action was of short duration, the disease quickly developing into its usual course and producing death. An even apparently beneficial action of subsequent injections was rare. Thus the serum was utterly powerless to stop an infection once well begun and to prevent the death of the animal.

INFLUENCE OF TREATMENT WITH A MIXTURE OF SODIUM OLEATE, IMMUNE SERUM, AND BORIC ACID.

In the experiments of this group the animal was first inoculated in the spinal canal. Afterwards, according particularly to the animal's physical appearance and the state of the spinal fluid, treatment was begun. As a rule, the treatment was repeated once a day during the life of the animal, or until the spinal fluid gave no, or very little, growth of pneumococcus. Each cubic centimeter of the mixture contained 0.1 of a cubic centimeter of a 1 per cent. aqueous solution of Merck's or Kahlbaum's sodium oleate, 0.2 of a cubic centimeter of the immune antipneumococcic serum, and 0.7 of a cubic centimeter of a 5 per cent. aqueous solution of boric acid. The mixture was always freshly prepared for each injection. In the protocols it is designated by the letters T. M. As in the experiments with immune serum, usually two cubic centimeters were injected each time during the early stages of the experiment, later it was often not possible to inject more than one cubic centimeter without causing symptoms of pressure. Of itself the mixture was harmless, both in infected animals and in normal ones employed to determine this point.

The nineteen experiments will be first recorded in the form of a table (table II) showing the gross features and result of each, after which a few that possess features of special importance or interest will be described at greater length.

From the table it is apparent that in those instances in which recovery took place it did so only after repeated injections of the mixture of sodium oleate, immune serum, and boric acid. In only one instance, that of monkey 28, were as few as three treatments given. The average number was five or six. This means that an actual disease was treated, as was clear from the animal's general

physical condition, its behavior, and from the continuous presence of pus and live pneumococci in the cerebrospinal fluid. After each treatment it was usual to notice an improvement in the physical condition, a clearing of the spinal fluid, and a reduction in the number of diplococci. This was true particularly of the first injection, which was usually followed by a disappearance of the bacteria from the circulating blood, and not only in those instances where recovery occurred but also often even in those which terminated fatally The subsiding of the bacteremia is probably due, as Dr. Flexner[11] has said in his lecture upon local specific treatment, not only to the control of the local infection but also to the action of immune principles which have diffused from the cerebrospinal fluid into the general circulation.

An example of the disease terminating in recovery is afforded by the protocol of monkey 35.

Sept. 26, 1911. Monkey 35. *Macacus rhesus;* fairly large.

4:05 P.M. L. p.: clear fluid; injected 0.1 c.c. of a 20 hour broth culture of X.11[1] (made directly from the heart's blood of a mouse). At 6:00 P.M. no change is apparent. Sept. 27, 9:00 A.M. Animal slightly ill; eyes watery and not so bright as yesterday; coat raised. 10:04 A.M., 18 hours after inoculation. L. p.: 2.5 c.c. of opalescent fluid quickly, under pressure. Films from the sediment show a few p. n. l. and about as many mononuclear cells; an occasional pair of extracellular diplococci. Culture gives moderate growth of pneumococcus.

Injected 2 c.c. of the mixture of sodium oleate, immune serum, and boric acid (T. M.) No symptoms. Throughout the remainder of the day the animal may be a little better.

Sept. 28, 9:00 A.M. Condition about the same; animal still moderately ill. 9:15 A.M. L. p.: 2 c.c. of turbid fluid freely; many p. n. l. and a few mononuclear cells; no cocci seen; no growth.

Injected 2 c.c. of T. M. Condition remains the same remainder of the day.

Sept. 29, 10:50 A.M. Animal better; coat still fluffy. L. p.: 1.3 c.c. of turbid fluid freely; a great many p. n. l. and many capsulated extracellular diplococci; an occasional pair intracellular; profuse growth. Injected 2 c.c. T. M. Sept. 30, 8:45 A.M. Condition same as yesterday. L. p.: 1 drop of turbid fluid; many p. n. l.; fewer diplococci, some involuted and Gram negative, and an occasional pair intracellular. Profuse growth but less than yesterday. Injected 1 c.c. of T. M. Oct. 1, A.M. Condition the same. 4:05 P. M. L. p.: 4 drops of slightly blood-stained fluid; few p. n. l., no cocci seen. Two colonies in the culture. Injected 2 c.c. of T. M. Oct. 2, A.M. Animal decidedly better; eats more and with some greed. P.M. Condition unchanged. Oct. 3. Better.

[11] Flexner, Simon, *Boston Med. and Surg. Jour.*, 1911, clxv, 709.

TABLE II.

Date.	Designation of animal.	Time elapsing between inoculation and beginning of treatment.	At the beginning of treatment.		Subsequent treatments.	Result.	Summary.
			Condition of animal.	Nature of spinal fluid.			
May 15, 1911	No. 22[9]	4 hrs.	Slightly ill; uneasy; cries; hypersensitive along spine.	None obtained; on the 2d day there were a few p.n.l. and extracellular cocci; moderate growth.	1, 2, and 3 days later.	Recovered.	Viable cocci were present in the spinal fluid for three days, during which time the animal was ill. Residual paralysis of right leg; complete recovery in five weeks.
May 23, 1911	No. 20[9]	6 hrs.	Quite ill; weak; crouches on perch; cries; coat erect.	A great many p.n.l. and extracellular cocci in pairs and short chains; profuse growth.	1 hr. later 5 c.c. of immune goat serum subcutaneously; 5 hrs. later another spinal treatment.	Died 31 hrs. after inoculation.	Undue susceptibility; marked reduction in number of bacteria followed the first injection, but none the second.
May 24, 1911	No. 28[9]	5 hrs.	Slightly ill; coat ruffled.	A few white blood cells mostly mononuclear; no cocci seen; scant growth.	23 and 29 hours later.	Recovered.	Many bacteria at the second injection; a few at the third, and none at the fourth puncture.
May 25, 1911	No. 29[9]	7 hrs.	Slightly ill; coat ruffled; hypersensitive along spine.	Great many p.n.l.; moderate number of extracellular diplococci. Moderate growth.	1 and 5 days later.	Died $5\frac{1}{3}$ days after inoculation.	Apparent recovery; "relapse"; sudden appearance of symptoms on fifth day followed quickly by death.
June 1, 1911	No. 30[9]	$4\frac{1}{2}$ hrs.	Quite ill; weak; torpid; hypersensitive.	Very few p.n.l. and extracellular diplococci; moderate growth.	24 and 30 hrs. later; also immune goat serum subcutaneously $4\frac{1}{2}$ and 24 hrs. after inoculation.	Died 33 hrs. after inoculation.	Offered very little resistance; treatment seemed without benefit.
June 1, 1911	No. 31[9]	$7\frac{1}{3}$ hrs.	Only slightly ill.	A few p.n.l. and extracellular diplococci; moderate	1, 2, 3, 5, and 6 days	Recovered.	Offered considerable resistance, yet an actual infection occurred and was arrested.

					ly 4½ and 24 hrs. after inoculation.		
June 1, 1911	No. 31[9]	7⅓ hrs.	Only slightly ill.	A few p.n.l. and extracellular diplococci; moderate growth.	1, 2, 3, 5, and 6 days later.	Recovered.	Offered considerable resistance, yet an actual infection occurred and was arrested.
Sept. 19, 1911	No. 33[10]	7 hrs.	Only slightly excited.	A few p.n.l. and extracellular diplococci; profuse growth.	1, 2, 3, and 4 days later.	Recovered.	Never very ill, but viable cocci in the spinal fluid for three days after inoculation.
Sept. 25, 1911	No. 34[10]	12 hrs.	Moderately ill, weak.	Few p.n.l. and extracellular diplococci; profuse growth.	1, 2, and 3 days later.	Died 11 days after inoculation.	Apparently well for one week after the fourth treatment. "Relapse" on eleventh day followed quickly by death.
Sept. 26, 1911	No. 35[10]	18 hrs.	Slightly ill.	2.5 c.c. under pressure; very few p.n.l. and small mononuclear cells; a few diplococci; moderate growth.	1, 2, 3, 4, and 9 days later.	Recovered.	Prolonged infection and illness of moderate degree; gradual recovery.
Oct. 10, 1911	No. 37[10]	24 hrs.	No symptoms of illness, although there is a bacteremia.	An occasional pair of atypical, extracellular diplococci; moderate growth.	1, 2, 3, 5, 6, and 7 days later.	Recovered.	Moderately ill for three days, during which time there were viable cocci in the spinal fluid and circulating blood. Then for a day improvement while the spinal fluid and blood gave no growth. Then worse, bacteria reappearing in the spinal fluid and blood. The second course of treatment was followed by permanent recovery.
Oct. 17, 1911	No. 38[10]	24 hrs.	More than slightly ill; bacteremia.	A few p.n.l.; no cocci seen; moderate growth.	1, 2, 3, and 5 days later.	Recovered.	The animal was moderately ill for 5 days. The bacteria disappeared from the blood after the 1st day and from the spinal fluid after the 3d. The last treatment was merely precautionary against recurrence.
Oct. 24, 1911	No. 39[10]	48 hrs.	Moderately ill; cries; coat raised; bacteremia.	An occasional p.n.l. and small mononuclear cell; no cocci seen; more than moderate growth.	1 and 3 days later.	Died 6⅔ days after inoculation.	After the first injection the bacteria disappeared from the blood and spinal fluid and the animal seemed well. On the fifth day it sickened, and until death breathed with great rapidity. At autopsy the meningitis was arrested; there was fibrinous pneumonia of the whole right lung, and septicemia.
Oct. 30, 1911	No. 40[10]	48 hrs.	Only slightly ill for 46 hrs.; then suddenly and severely	Many p.n.l.; and a great many cellular diplococci; very profuse growth.	None.	Died 53 hrs. after inoculation.	Delayed onset of fulminating illness. Animal moribund when first treated. At autopsy enormous reduction in the number of bacteria in the spinal fluid was found to have taken place; followed one injection of serum.

			very ill; prostrate; bacteremia for two days.				
Nov. 6, 1911	No. 41[10]	45 hrs.	Moderately ill; bacteremia for two days.	Many p.n.l.; a few extracellular diplococci; slight phagocytosis; profuse growth.	1, 3, 4, 5, 10, 11, and 12, days later.	Died 14 days after inoculation.	Irregular course. The intermission in the treatment corresponded to days of great improvement and failure to obtain cultures from the spinal fluid. Marked anemia and emaciation during the second week. Autopsy: cerebral meninges intensely inflamed, the spinal slightly.
Nov. 27, 1911	No. 43[10]	20 hrs.	Extremely ill, and rapidly becoming worse; septicemia.	Enormous numbers of p.n.l.; myriad diplococci nearly all extracellular; very profuse growth.	None.	Died 22½ hrs. after inoculation.	Inoculated in the evening and found moribund the next morning.
Dec. 5, 1911	No. 44[10]	20 hrs.	Slightly ill; bacteremia.	Fluid coagulates great many p.n.l., very great many diplococci extracellular; profuse growth.	1, 2, 2¼, 3, 4, 5, and 6 days later.	Recovered.	Much improvement after first treatment; then slow progress to complete recovery in ten days.
Dec. 14, 1911	No. 46[10]	16 hrs.	Gravely ill; septicemia.	Many p.n.l.; myriad extracellular diplococci; very profuse growth.	2 and 5 hours later.	Died 1⅔ days after inoculation.	Great destruction of bacteria and marked general improvement followed the first treatment, but bacteria soon multiplied. Little restraint by the 2d and 3d treatments. Inflammation most intense in cranium. Septicemia.
Jan. 8, 1912	No. 49[10]	18 hrs.	Very ill; sits motionless; cannot be roused; bacteremia.	Very many p.n.l., myriad cocci in pairs and short chains; occasional phagocytosis.	4 hours and 1 day later.	Died 2¾ days after inoculation.	The first treatment was followed by improvement which lasted a few hours. Septicemia; dry peritonitis.
Jan. 17, 1912	No. 51[10]	8⅙ hrs.	Moderately ill; lies down at times.	Very many p.n.l., few extracellular diplococci; profuse growth.	15 hours and 2, 3, 4, 6, and 12 days later.	Recovered.	Improved steadily after the first treatment. At the 6th treatment the spinal fluid was sterile. Animal well until the 12th day, when it became a little excited. L. p. gave clear sterile fluid, but a precautionary treatment was given. From the next day on animal perfectly well.

[9] The therapeutic mixture contained immune goat serum. [10] The therapeutic mixture contained immune horse serum.

Oct. 4. Still improving. Oct. 6. Animal well. Yet fearing a possible relapse (compare No. 34) l. p. was done at 4:00 P.M. 3 drops of blood-tinged fluid; no excess of p. n. l. over normal ratio to red blood cells; no cocci seen; two colonies in culture. Injected 1 c.c. of T. M.

Nov. 5. Observed daily, animal has shown no signs of illness until today when there is diarrhea. Nov. 7. Diarrhea worse; emaciation. Nov. 10. Animal found dead this morning. Autopsy at once: moderate emaciation. Nothing abnormal found in the posterior cavity. There is an acute colitis of severe degree; hyperplasia of the mesenteric lymph nodes.

Upon microscopic examination nothing abnormal is found in the spinal cord or its membranes.

In the experiments which terminated fatally the disease followed one of two well characterized and readily recognizable courses. Either the animal was gravely ill when the treatment was begun and died during the first or second day, nearly always from septicemia; or the animal was not so ill at the beginning of treatment, the customary improvement followed the first injections, recovery seemed to have taken place, the treatment was discontinued, and then later what may be designated a relapse occurred and was quickly followed by death. In one instance (protocol 39), after apparent recovery a massive fibrinous pneumonia, instead of a relapse, developed and led to death. The meningitis was healed.

The first type of fatal disease is illustrated by the protocol of monkey 43.

Nov. 27, 1911. Monkey 43. *Macacus rhesus;* medium size.

1:40 P.M. L. p.: clear fluid; injected 0.1 c.c. of a 24 hour broth culture of X.11[2]. No evidences of illness during remainder of day. Nov. 28, 8:00 A.M. Animal quite ill. 9:15 A.M. Very ill. 9:35 A.M. Seems worse than twenty minutes ago; very weak; can no longer sit up. L. p.: 1.3 c.c. of very turbid fluid containing flocculi; very many p. n. l. mostly poorly staining and an enormous number of diplococci nearly all extracellular. Very profuse growth. Likewise profuse growth from four drops of blood taken from the ear. Leucocytosis about 25,000. Injected 1.5 c.c. of T. M. No immediate change in the animal's condition, but it steadily weakens. Death at 12:15 P.M., 22½ hours after inoculation.

Autopsy.—1:30 P.M. Slight congestion of spinal meninges. There is a scant, sticky, yellowish white exudate all along the surface of the pia. It contains many p. n. l. and still a great many diplococci but not nearly so many as were in the fluid drawn off prior to the treatment. Extreme phagocytosis has occurred. Most cocci are within pus cells and many pus cells are crowded with the organisms. Cultures furnish moderate growth. The cerebral pia is more congested than the spinal, and there is more exudate. In the heart's blood are a few diplococci. Profuse growth occurs from it.

The second type of fatal disease, that of apparent recovery followed, upon discontinuation of treatment, by late relapse and death, is illustrated by the protocol of monkey 29.

May 25, 1911. Monkey 29. *Macacus rhesus;* medium size.

10:50 A.M. L. p.: 1 c.c. of clear fluid; injected 0.1 c.c. of a 24 hour broth culture of X.5[16]. 2:15 P.M. Animal uneasy. 5:30 P.M., 7 hours after inoculation. Animal slightly ill; hypersensitive along spine; coat ruffled. L. p.: 2 c.c. of turbid fluid; many p. n. l., an occasional pair of cocci extracellular; more than moderate growth. Injected 2 c.c. of T. M. May 26, 10:30 A. M. Somewhat better. L. p.: 2 c.c. of turbid fluid; more p. n. l. than yesterday; only one pair of diplococci seen; a single colony grows from the transplant. Injected 2 c.c. of T. M. Condition remains the same during remainder of day. May 27, A.M. Much better; animal springs up and down as normally. May 28. Well. May 29. Still seems well.

May 30, 9:20 A.M. The animal is found with coat slightly raised, sitting on its perch. It cannot be made to come down; cries when disturbed. Made many attempts, in several spaces, to obtain spinal fluid; all failed; even three made under light ether anesthesia. Finally, injected with little resistance 1.5 c.c. of T. M.; no pressure symptoms observed. The animal quickly recovers from the ether and upon being put into its cage climbs upon the perch and remains sitting as before. 2:00 P.M. Still sits upon perch; seems about the same. 4:30 P.M. Animal dead. Death occurred $5\frac{1}{2}$ days after inoculation.

Autopsy.—May 31, 9:30 A.M. The spinal cord beneath the dura is enveloped throughout its length by a thick, yellowish white, translucent sheath comprising the pia infiltrated and covered by a fibrinous exudate, the whole averaging from 1 to 2 mm. in thickness. Films show many p. n. l. in all stages of disintegration, and an occasional coccus, but so few that only after a search of several minutes are a few swollen pairs found within a single pus cell.

The pia of both cerebral hemispheres and to a less extent that of the cerebellum is finely congested and studded with abundant punctate hemorrhages. Films from the cerebral cortex contain many well preserved p. n. l. and many typical extracellular diplococci. There is less similar exudate at the base of the brain. The ventral cavity of the body is negative. Cultures from the exudate in the lumbar and thoracic regions of the spinal cord and from the heart's blood remain sterile. From the right cerebral cortex the pneumococcus grows abundantly and pure.

In this experiment the effect of the injections in almost sterilizing the spinal canal is apparent. The clinical "relapse" and death of the animal were evidently due to the progress of the inflammation in the cranial cavity which had been cut off from communication with the spinal subdural space by the fibrinous exudate.

DISCUSSION.

The experimental data presented have more than a theoretical interest. They should indeed be considered with respect to their application to the treatment of a highly fatal disease of human beings. Pneumococcic meningitis is far from being a rare affection. and the number of reported recoveries from the disease is very small. There exists at present no effective treatment that has come to be at all generally employed. The few instances in which antipneumococcic serum has been applied have not yielded results that inspire confidence in its sole employment. Even when administered by direct intraspinal injection the effects have been doubtful. Matthes[12] so treated three patients and all died; Grober,[13] two, one of which recovered and one died; and Schlesinger[14] and Kleinschmidt,[15] one each, both recovering. The total number is small, and although three of seven treated with serum recovered there have been reported ten more instances of recovery following no more radical treatment than mere lumbar puncture and withdrawal of fluid.[16] The reasons for the inefficiency of serum treatment are now rendered fairly clear. An antipneumococcus serum is at best active only against the homologous organism or organisms the types of which have been employed in its preparation. Under optimal experimental conditions the extent of its efficacy is confined within a brief space of time; and in general this will probably be found equally true of the so called spontaneous human pneumococcus meningitis. The outlook for its successful employment alone in the human disease is not encouraging.

The therapeutic mixture of sodium oleate, boric acid, and antiserum holds out greater promise. The scope of its efficacy is far wider than that of the antiserum alone. It remains still to be determined whether its action is as closely restricted within the limits of homologous strain of pneumococcus as is that of the anti-

[12] Matthes, M., *Med. Klin.*, 1908, iv, 733.

[13] Grober, J., *München. med. Wchnschr.*, 1910, lvii, 1332.

[14] Schlesinger, H., *Wien. med. Wchnschr.*, 1911, lxi, 40.

[15] Kleinschmidt, *Med. Klin.*, 1911, vii, 1195.

[16] Rolly, Fr., *Deutsch. med. Wchnschr.*, 1911, xxxvii, 774; Kleinschmidt, *loc. cit.*

serum. This point, which is of high importance, is under examination at present so that a decision should soon be reached.[17]

Fortunately the number of strains or types of pneumococci appears not to be large. Neufeld and Händel[18] have noted that most strains of pneumococcus are subject to the action of an immune serum prepared with a single culture of their type I pneumococcus, which is the culture employed for immunizing the goat and horse used in these experiments. Neufeld and Händel recognize four serum-specific types of pneumococcus, three of which are infrequent. It may happen that the specificity is not absolute with respect to the oleate serum mixture, and it is also, of course, possible that the obstacle to the usefulness of a single immune serum may be obviated by preparing a suitable polyvalent serum.

Other considerations which may affect the results of the employment of the oleate serum mixture are clinical in nature. From the experiments it appears that pneumococcic infections, started in the region of the brain, follow a severer course, and are less amenable to treatment than those started in the region of the spinal cord. In both, the experimental procedures produce traumatic injury and this injury gives to the infection a graver character when inflicted on the tissues of the brain than when inflicted on those of the cord. A class of pneumococcic inflammation of the meninges in man arises from infections of the membranes about the brain; namely, in middle ear disease, fracture of the base of the skull, infection of the accessory air sinuses, operations on the nasal tissues, etc. It remains to be ascertained to what extent this is a determining factor in the control of natural pneumococcus meningitis.

A great number of cases of pneumococcus meningitis in man follow blood infection with the pneumococci and are secondary to in-

[17] Tentative experiments performed on rats and mice with two cultures of atypical strains of pneumococcus, obtained from the circulating blood of pneumonic patients, indicate that the atypical strains stand midway between the highly soluble typical pneumococcus and the insoluble *Streptococcus pyogenes* in respect to solubility in sodium oleate. The experiments indicate further that the atypical strains are resistant to the action of the mixture of typical antiserum, sodium oleate, and boric acid. Experiments are in progress to determine the effect of polyvalent immune sera which carry the specific antibodies for the atypical strains.

[18] Neufeld, F., and Händel, *Ztschr. f. Immunitätsforsch., Orig.*, 1909, iii, 159; *Arb. a. d. k. Gsndhtsamte*, 1910, xxxiv, 293.

flammations of the lung, pleura and heart, and arise generally throughout the meninges. In these cases the therapeutic control of the meningitis need not be equivalent to the suppression of the pathological conditions as a whole, to which the patient may still succumb. On the other hand, it is often just the concurrent meningitic infection that makes the general clinical conditions grave, so that with its abatement the outlook for the cessation of the other inflammation becomes much improved. Fortunately, the direct attack upon the meningitic infection is capable of affecting the blood infection since the eruption of pneumococci from the meninges into the blood is arrested while the ready escape of the therapeutic mixture from the meninges into the blood[19] provides a favorable condition for the action of the immune serum upon the pneumococci already present in the blood, as well, possibly, as those situated in the interior of some organs.

The experiments show that the time interval between onset of infection and beginning of the specific treatment is a very important factor. The reasons for this are several: the degree of the infection and attending intoxication; the numbers of multiplying diplococci; the nature of the inflammatory exudate,—whether serous, purulent, or fibrinous,—that effects the penetration of the mixture to the diplococci and the remote parts of the cerebrospinal meninges. A purulent and fibrinous exudate is not only permeated less readily by the mixture, but its high protein content tends to reduce the action of the oleate upon the pneumococci upon which the efficiency of the mixture so much depends. Hence serous or seropurulent inflammations will probably be more subject to control than purulent and fibrinous ones. And yet in practice this distinction may make less difference than in the experiments in which the course of the infection is abnormally rapid. Should it be found that infection of the spinal meninges is sometimes controlled, while that of the cerebral meninges and ventricles is not, it will be well to consider the injection of the mixture directly into the cerebral ventricles, as has been successfully done in the serum treatment of epidemic meningitis.[20]

[19] Flexner, Simon, *loc. cit.*

[20] Cushing, H., and Sladen, F. J., *Jour. Exper. Med.*, 1908, x, 548; Fischer, L., *N. Y. Med. Jour.*, 1910, xci, 625.

Finally, the specific treatment described is not necessarily confined to meningitis but may be applicable to other local pneumococcic infections, such as those of the pleura, joints, and possibly the peritoneum. The determining conditions will be similar: the type of pneumococcus, the accessibility of the focus of infection (whether isolated by adhesions or communicating with the cavity into which the mixture is injected), the nature of the inflammatory exudate, and the coëxistence of other slight or severe infections in inaccessible organs or parts. The oleate soap obviously possesses higher affinity or greater avidity for certain bacteria (e. g., pneumococcus) than for protein in general, since the boric acid suffices to hold it apart from serum protein under conditions in which it still attacks the bacteria.

CONCLUSIONS.

Virulent pneumococci injected into the cranial or spinal cavities of monkeys produce constantly a meningitis closely resembling pneumococcus meningitis in man, except that the experimental disease pursues a more rapid course to the invariable death of the untreated animal.

An homologous immune pneumococcus serum injected into the spinal canal exerts a restraining influence upon the disease to the extent that when employed early it prevented, exceptionally, the occurrence of infection and thus saved the life of the animal, and when given later it at first retarded the disease but subsequently exerted no beneficial action and was powerless to save life.

A mixture of sodium oleate, immune serum, and boric acid exerted regularly a more powerful action than immune serum alone, and not only prevented the occurrence of infection but also, when administered repeatedly, arrested the progress of an actually established infection and led, often, to the enduring and perfect recovery of the inoculated animal.

It is proposed to employ a similar mixture in the direct treatment of pneumococcic meningitis and possibly of still other accessible local pneumococcic infections in man.

PIGMENT FORMATION IN THE LIVER DURING AUTOLYSIS AND ITS RELATION TO THE PIGMENTATION OF HEMOCHROMATOSIS.*

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While engaged in studying the problems presented by the disease known as hemochromatosis, a recent paper of Brown (1) impressed us with the possibility that the knowledge of autolytic changes might be of aid in the explanation of the pigmentation occurring in this and some other diseases.

Several authors (2) have insisted that hemosiderin occurs in the parenchymatous cells of the liver, especially in those cells which show some evidence of degeneration. Particularly is this the case in hemochromatosis in which there is an enormous production of an iron-containing pigment in the parenchymatous viscera, especially in the liver and pancreas. In the liver there may be seen all stages from the cell in a fair state of preservation with a few granules to the cell whose membrane seems stretched by the load of pigment and whose nucleus is barely perceptible. Still other masses of pigment occur about which no cell membrane can be recognized, but which in size and general appearance resemble the heavily loaded liver cells. Thus through cell degeneration and irritation of the interstitial tissues through the deposit in them of pigment, there arises a productive inflammatory process which results in a fibrosis of the organ. Similar processes, although less marked, take place in other viscera.

The understanding of the disease, the cardinal features of which are the general pigmentation, cirrhosis of the liver, and diabetes, lies in the explanation of the pigment production since most of the evidence at hand points to this condition as the primary event. Inasmuch as most of the pigment contains iron in an easily

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demonstrable form, it has been generally assumed that it had its origin in the hemoglobin of the red blood cells, and a primary disease of the blood or some alteration of the blood associated with excessive intravascular destruction of the red blood cells has been invoked to explain the unusual accumulation of hemosiderin.

One of us (3) has recently discussed this question at some length and taken the view that there is little justification for the assumption of a primary blood dyscrasia. In those cases in which records of blood studies are available, we find either normal pictures or a slight secondary anemia which might well be expected considering the condition of the patients. Examination of the bone-marrow in the cases reported from this laboratory showed no evidence of unusually active hematopoiesis. Furthermore, the distribution of the pigment in hemochromatosis greatly exceeds and varies widely from that found in pernicious anemia in which the pigmentation is universally ascribed to blood destruction. Finally, we cannot consider as due to hemolysis a striking feature in hemochromatosis, the accumulation in several organs of pigments which have no relation to hemoglobin.

The alternative suggested is that of a widespread simultaneous parenchymatous cell degeneration of a special nature affecting many organs leading to the deposition of a variety of pigments, the composition of the pigment in any one cell being determined by the chemical processes going on there. The iron of the hemosiderin we must refer to the so called masked, organic or tissue iron which we have reason to believe occurs in especially large quantities in those cells which characteristically are the seats of the hemosiderin deposits (4). The tissue iron must be replenished, doubtless, by the blood, but we believe it has been shown in the above mentioned paper (3) that this may be done by the iron derived from the physiological dissolution of the red cells. Such an explanation calls for a marked iron retention in the progressive stages of hemochromatosis, a point at present under investigation.

Brown has reported upon the pigmentary changes in the rabbit liver taking place during autolysis in moist chambers under aseptic conditions. The exuded fluid from the autolyzing tissue was studied and small pieces of tissue were removed at intervals and fixed in alcohol. He reports hemosiderin as of prac-

tically constant occurrence in the liver, and that during about 48 hours of autolysis there was a definite increase in the granular hemosiderin of the liver cells and in the formation of masses of hemosiderin in the capillaries and larger vessels. Coincident with or following close upon this increase, there was an increase in the hematoidin in the fluid and the development in the liver cells of an iron-free pigment partially soluble in chloroform and alcohol, in which solvents hematoidin and other bile pigments could then be demonstrated. Finally, the hemosiderin of the liver cells was observed to lose the iron reaction as the nuclei underwent dissolution, and large amounts of colorless and pigmented amorphous and crystalline material, containing both phosphorus and iron, appeared throughout the section, but more particularly in the portal spaces. The fact that the increase in pigmentation took place best at a temperature of 37° C. and near the exposed surfaces suggests that hemosiderin is "an oxidation product of hemoglobin due to enzyme action." This is the first instance in which hemosiderin has been shown to be formed outside the living animal body.

This method of studying pigmentary changes in organs by submitting them to autolysis offers an opportunity of testing one of the disputed features of hemochromatosis which we have mentioned; namely, the possibility of the formation of hemosiderin within parenchymatous cells in the absence of blood. Accordingly we undertook a series of experiments in which the rabbit liver was first thoroughly perfused with Locke's solution and then placed in a moist chamber under aseptic conditions and allowed to undergo autolysis.

EXPERIMENTAL PART.

Method.—The rabbit's abdomen was first opened, a clamp placed at the hilum of one lobe of the liver to prevent the perfusion of that lobe, and the abdominal incision temporarily closed. A jugular vein and carotid artery were next exposed, a cannula was inserted in the jugular vein, a nick made in the carotid artery and the perfusion begun with sterile, oxygenated Locke's solution. The operations were performed under aseptic conditions. When the effluent from the carotid became clear, the cannula was inserted into the portal vein and the hepatic vein above the liver severed. At the end of the perfusion the liver, excepting the portion previously clamped off, appeared entirely bloodless although in some cases a few red blood cells were found on microscopic examination.

The liver was then removed, a small piece taken from both perfused and non-perfused portions, and fixed at once in 90 per cent. alcohol.

The rest was placed on small glass stands in large sterile Petri dishes, 23 cm. in diameter and 8 cm. deep. A small dish of water and one containing toluol were placed on the floor of the larger vessel. Oxygen was passed into the vessels through a small tube four or five times during the day. Two vessels were used for each rabbit, usually one standing at room temperature and the

other placed in the thermostat at 37° C. In some experiments one or both of these moist chambers were placed in direct sunlight. Small pieces were removed at intervals and fixed in 90 per cent. alcohol and in formalin. The alcohol-fixed tissues were embedded in celloidin and three sets of sections prepared, one unstained, one stained in hematoxylin and eosin, and a third treated by Nishimura's method for iron. This method, which has given us the best microchemical results, consists in immersing the sections in ammonium sulphide for one hour, washing in water, treating with a mixture of equal parts of 2 per cent. potassium ferrocyanid and 1 per cent. hydrochloric acid for 20 minutes, washing in warm ½ per cent. hydrochloric acid and then thoroughly in distilled water.

Results.—The changes occurring during autolysis have been well discussed by Brown. We have been able to confirm many of the points in his report, while some few points of difference will be mentioned. We have not followed the later stages, confining our studies to the alteration occurring during the first two or three days.

The change in the color of the tissues when examined grossly is often striking and is limited, as Brown pointed out, to the exposed surfaces. When the liver rests upon a glass slide through which the light strikes it from below, the color of this surface is much paler than of parts exposed to the air. Diffuse daylight has little effect in hastening the color changes, a temperature of 37° C. in a dark thermostat being more effective.

The most rapid and striking changes, however, occur in direct sunlight. Within a very short time the darkening may be noted and especially along the edges and angles of the tissue. After several hours' exposure most of the surface of the perfused liver becomes a much darker brown while that of the blood-containing lobe is often almost black. Cross section reveals a border 2 or 3 mm. thick which is darker, dryer, and apparently much more condensed than the central part. These changes in direct sunlight occur almost as quickly when the dishes are packed in ice to prevent elevation of the temperature.

In his histological description of the alcohol-fixed tissue, Brown makes use of certain terms which it may be convenient for us to follow. He states that a section of liver from an average animal, fixed in alcohol, will show the following: "(1) A peripheral zone of pigmented material reacting for iron and containing granular, pigmented masses and patches of pigment which do not react for iron. (2) A zone of condensation with granular and diffuse pigmentation of all elements which react decidedly for iron as well as granular pigment which does not react. (3) The depths of the section show a few granules of hemosiderin and a few granules of bile pigment in the liver cells, the cells of Kupfer, and the leucocytes. (4) The tissue elements do not react for iron."

In tissues which have undergone autolytic changes he speaks further (5) of the "development of a zone beneath all exposed surfaces in which all changes are most pronounced and which might be termed the zone of reaction."

These different zones are readily recognized, but we do not find

a constant iron reaction in the peripheral and condensed zones, nor do we find in most cases hemosiderin present in the liver cells of the rabbits. In fact in only four of fifteen rabbits was hemosiderin present in appreciable amounts. In most of the others there were granules of pigment of similar appearance and distribution, which failed, however, to give an iron reaction even with prolonged treatment with ammonium sulphide, or after preliminary treatment with hydrogen peroxide. These pigments whether iron-containing or not were usually increased during autolysis both in the perfused and non-perfused liver tissue. In three cases there was very definite increase in the amount of hemosiderin in the cells of the perfused liver, especially well seen in the zone of reaction just beneath the exposed surfaces. No distinct difference was noted in these specimens at the end of twenty-four hours of autolysis at room temperature, but at 37° C. the changes during this period were indicated by the increased size and number of the hemosiderin granules throughout the section. In specimen 4, for example, the increase was very definitely shown at the end of seventy hours at 37° C., when in sections stained for iron, the zone of reaction stood out as a broad blue band beneath the surface easily visible to the naked eye. Its prominence was doubtless due in part to the shrinkage of the tissues and the consequent concentration of the increased amount of pigment into a smaller space. This zone is shown by the microscope filled with pigment granules which react for iron and also stain darkly with hematoxylin. In unstained sections they appear as bright yellow granules of various sizes. In the depths of the section, at this stage, the pigment is less conspicuous than in the control tissue fixed immediately after the animal's death.

In only one instance was hemosiderin formed during the autolysis of a liver in which it was not found in the tissue fixed immediately after the death of the animal, and this occurred in the lobe containing blood. The increase in intracellular iron-containing pigment seemed almost, if not quite as great in the perfused as in the blood-containing portion of the same liver.

In several instances the iron-free pigment was increased during autolysis and often to a greater extent in the perfused than in the blood-containing lobe. The pigment disappears under the influence

of alkalis and more slowly in hydrogen peroxide. It is not readily soluble in mineral acids. Fat stains and fat solvents have no apparent effect upon it, and we are inclined to consider it analogous to that found in the so called brown atrophy of the liver, in the reticular zone of the adrenal cortex, and in the cardiac muscle fibers, a product presumably of the proteid constituents of the cell in which it occurs.

SUMMARY.

Without claiming any close analogy between the autolytic changes and pigmentation in pathological processes, it has, we believe, been shown that iron-containing, as well as other kinds of pigments, may be formed during the autolytic degeneration of parenchymatous cells independently of the hemoglobin in the blood stream, and hence may be derived from the proteid constituents of the cell itself.

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NERVE FIBRILS IN THE SINO–AURICULAR NODE.*

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Plates 65–67.

The purpose of the following paper is to describe nervous structures found within the sino-auricular node by the use of a vital stain of methylene-blue. Since the original description by Keith and Flack (1) in 1907, considerable interest has attached to the sino-auricular node; and its normal histology, its function, and its condition in pathological hearts have been actively investigated. As regards its function, the experimental evidence points strongly to it as the *primum movens* of the heart (2, 3, 4), although a very few observers apparently are not inclined to accept this view as yet (5, 6, 7). Its relation to certain clinical conditions, more especially to auricular fibrillation (pulsus irregularis perpetuus), is being studied, but so far the reports on the hearts examined consist in the main of descriptions of the findings in and around the node without any assertion that the changes in the node account for the clinical condition.

Pathological changes of the intranodal nervous structures have not received much attention; indeed thus far no one has described the finer nervous elements occurring normally within the node. In addition to the numerous studies of the general cardiac nervous system, both intra- and extracardiac, which have extended over many years, three recent important contributions to the nervous structures of the auriculo-ventricular system have been made; namely, those of Wilson (8), De Witt (9), and Engel (10). Wilson was the first to demonstrate by means of a vital stain not only

* A brief report of this work was presented at the meeting of the New York Pathological Society, January, 1912. (*Proc. N. Y. Path. Soc.*, 1912, xii, 103.)
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the nerve fibers accompanying the bundle of His, but also ganglion cells of various types and a rich plexus of nerve fibrils intimately related to the specialized musculature of the conducting system. The bundle of His may therefore be considered anatomically a neuromuscular structure. As the region of the sino-auricular node is now considered by many the pace-maker of the mammalian heart, we have thought it of importance to make a similar investigation of its nervous elements.

The study described in the present paper has been confined to this narrow specialized structure, the sino-auricular node, and its immediate environment. As to its connection with extracardiac nerves Keith and Flack originally stated that the nerves supplying this region are the vagus and the sympathetic; and in 1910 Flack (11) added that "at this spot the vagus and sympathetic nerves, particularly the right nerves, exert their main effect upon the heart rhythm." Argaud (12) found that the nervous supply of the sino-auricular region has its origin in a plexus of nerves situated at the root of the right coronary artery as it arises from the aorta; incidentally we may mention that he considers this system a coördinating mechanism connecting the beginning and end of the heart beat. The intimate relation of the sino-auricular node with the subepicardial nerves and ganglia overlying it was particularly emphasized by Keith and Flack and will be evident to anyone examining this region; and in the literature there is even an occasional mention of a nerve fiber or ganglion cell within the limits of the node (Koch (13), and Schwartz (14)). But no one thus far has used a specialized nerve stain for the Keith-Flack node and consequently there has been no description whatever of nerve fibrils.

For the demonstration of the nerve fibrils and ganglion cells within the node we employed a vital stain of methylene-blue, following for the Keith-Flack node the same technique as Wilson (15, 16) applied to the bundle of His. In the course of the work we also had the advantage of having Dr. Wilson personally demonstrate the procedure to us.

In detail, this method was as follows:

(*a*) With a double knife long pieces, not more than 1 mm. thick, were cut along the posterior left aspect of the crista terminalis of His. It is impossible

to dissect out grossly the sino-auricular node with anything approaching the certainty with which the auriculo-ventricular bundle can be isolated.

(*b*) These pieces were immersed in a very weak solution of methylene-blue; namely, the methylene-blue "nach Ehrlich" prepared by Grübler. The pieces were left immersed, epicardium up, in this dilute methylene-blue solution at 37° C. for about ten minutes (until they just became permeated with the dye).

(*c*) Next the specimens were passed through 0.8 per cent. salt solution at 37° C.

(*d*) The salt solution was poured off except for the moisture at the bottom of the dish and the tissue (epicardium up) was aërated in the oven at 37° C. Until the nerve elements were stained blue, microscopic examination of one piece of tissue after the other was made. The appearance of the methylene-blue in the nerve fibrils occurred after from 10 to 60 minutes' aëration of the tissues employed.

(*e*) As soon as the nerve elements were stained, the specimen was fixed in 8 per cent. ammonium molybdate. The large crystalline variety of ammonium molybdate prepared by Merck was added to distilled water, and the mixture heated, but not allowed to boil, until the crystals were dissolved. This solution was put into the ice chest before dropping the stained tissue into it. The specimens were left over night in a large amount of the cold fixative.

(*f*) The following morning the objects were washed in ice cold running tap water for half an hour.

(*g*) Next the pieces were laid for a few seconds on filter paper.

(*h*) Then they were passed once quickly through cold 96 per cent. alcohol and then through four changes of cold 96 per cent. alcohol, 15 minutes in each.

(*i*) Thereupon the pieces were carried into absolute alcohol still kept in the ice chest. Altogether they were left in absolute alcohol for one hour, with three changes. The last change of absolute alcohol was at room temperature.

(*j*) The tissue was placed in xylol. The clearing of the objects took about half an hour.

(*k*) Finally the specimens were put into paraffin *in vacuo* for one hour, embedded, cut into sections varying from 15 to 80 microns in thickness, and mounted in Canada balsam.

As is usual with methylene-blue, the method was not uniformly successful either in different specimens or in different parts of the same specimen. Occasionally the sections were counterstained with alum-carmine. Neither the methods of Cajal nor Golgi were attempted.

After staining with methylene-blue, even without counterstaining, the sino-auricular node is recognized by its location immediately under the epicardium, by the syncytial network which characterizes its musculature, the finer size of the individual muscle fibers, their vacuolated appearance, and finally by the indistinctness of their cross striations (figures 1 and 2). We have been especially careful to make certain that the nervous elements about to be described as

within the limits of the node have been in the midst of the nodal musculature as just defined and not in the subepicardium. The fine fibrils with their varicosities were distinguished from connective and elastic tissue fibrils and also from delicate capillaries.

The hearts used in this investigation have been those of the pig, sheep, calf and bullock, dog, and to a less extent, the heart of man. The nerve elements studied in these hearts were: (1) nerve fibers; (2) nerve fibrils associated with the Keith-Flack nodal musculature; (3) nerve fibrils associated with blood-vessels; (4) ganglionic cells; (5) nerve fibrils associated with the ordinary auricular myocardium immediately surrounding the sino-auricular node.

The subepicardial nerves were found to send branches into the interstices of the nodal musculature in the hearts of man, dog, and of the hoofed animals investigated. In the pig, sheep, bullock, and calf, we found a plexus of nerve fibrils in the sino-auricular node such as Wilson described for the auriculo-ventricular system (figures 1, 2, and 3). Fine neurofibrils with varicosities were seen intimately interwoven with the specialized musculature of the Keith-Flack node, and were also seen surrounding the special nutrient artery of the node. Such fibrils were identical in appearance with those which ran lengthwise throughout the nerve fibers in more or less parallel wavy lines. Here, as described by Wilson for the auriculo-ventricular system, what we observed was not a single nerve fiber breaking up and surrounding a single muscle fiber, but a complicated network surrounding alike single muscle fibers and groups of fibers. We have seen no undoubted nerve endings on the individual muscle fibers, at least nothing so definite as the brush-like nerve terminations which we found about the subepicardial ganglionic cells in the sheep, steer, and dog. In figure 3, from the sino-auricular node of a bullock, a coarser, long, moniliform fibril is apparently breaking up into finer varicose branches which as usual are closely applied to the nodal muscle fibers. A plexus of fine nerve fibrils was found in the wall of the nutrient artery of the node similar to that surrounding its muscle fibers, not only in the pig, sheep, and calf, but also in the dog. If we accept the distinction made by Dogiel (17), these fibers in the wall of the artery were vasomotor and not sensory, as they were fine in structure and

plexiform in arrangement, whereas the sensory fibrils supplying vessel walls are described as coarser in structure and arborescent in arrangement.

In the pig's heart there occurred in addition to these nerve fibrils a multipolar ganglionic cell, and mention is made by Lewis (18) of ganglion cells (stained with Van Gieson's solution) embedded within the node of the pig in a section presented to him by Dr. Ivy Mackenzie. However, although our sections stained with cresyl violet showed numerous subepicardial ganglion cells in the pig and dog with striking clearness, they did not demonstrate any ganglion cells within the node. In the sino-auricular node of the human heart only an occasional intranodal ganglion cell was seen in sections stained with Van Gieson's picric acid fuchsin. In brief, then, the fact that we found so few ganglionic cells in the node of human and pig hearts, and none whatever in the hearts of sheep, bullock, and dog, may perhaps be ascribed to a lack of ganglion cells and not to the technique employed, as we encountered no difficulty in demonstrating the subepicardial ganglia in all our sections.

This paucity is in striking contrast to the wealth of ganglion cells as described by Wilson and by Engel for the auriculo-ventricular bundle.

Wilson and Engel evidently found it more difficult to demonstrate the neurofibrils in the auriculo-ventricular system in dog and man than in the ungulates. We encountered a similar difficulty in the sino-auricular node. Our failure to discover neurofibrils in the sino-auricular node in man may be accounted for by our inability to secure fresh human material, but in the dog we had fresh hearts and yet were successful in demonstrating only vasomotor fibers and not an intermuscular plexus of fibrils.

It is not intended to assert or deny in the foregoing description that the nervous structures in the sino-auricular node have a function independent of that of the plexus supplying the ordinary auricular myocardium. Indeed, histologically we could find no difference in structure between the two, as evidence of a possible difference in function; but at times (perhaps owing to the capriciousness of methylene-blue) we noticed that the nervous plexus in the specialized tissue was well stained when that in the surrounding

myocardium was little or not at all stained. And this latter observation corresponds with a distinction observed by De Witt in regard to the nerve supply of the auriculo-ventricular system as compared with that of its environment. It may well be that the neurofibrillar plexus represents the distribution of the extracardiac nerves, vagus and sympathetic, to the sino-auricular node, and does not differ in the character of its function from the plexus of the surrounding auricular musculature.

SUMMARY.

Aside from the nerve fibers which enter the pace-making node from the subepicardium, there were observed within the limits of the node a few ganglionic cells and a plexus of moniliform nerve fibrils in very close relation to its muscle fibers, hence the sino-auricular node, like the auriculo-ventricular, may be considered from a histological standpoint a neuromuscular organ.

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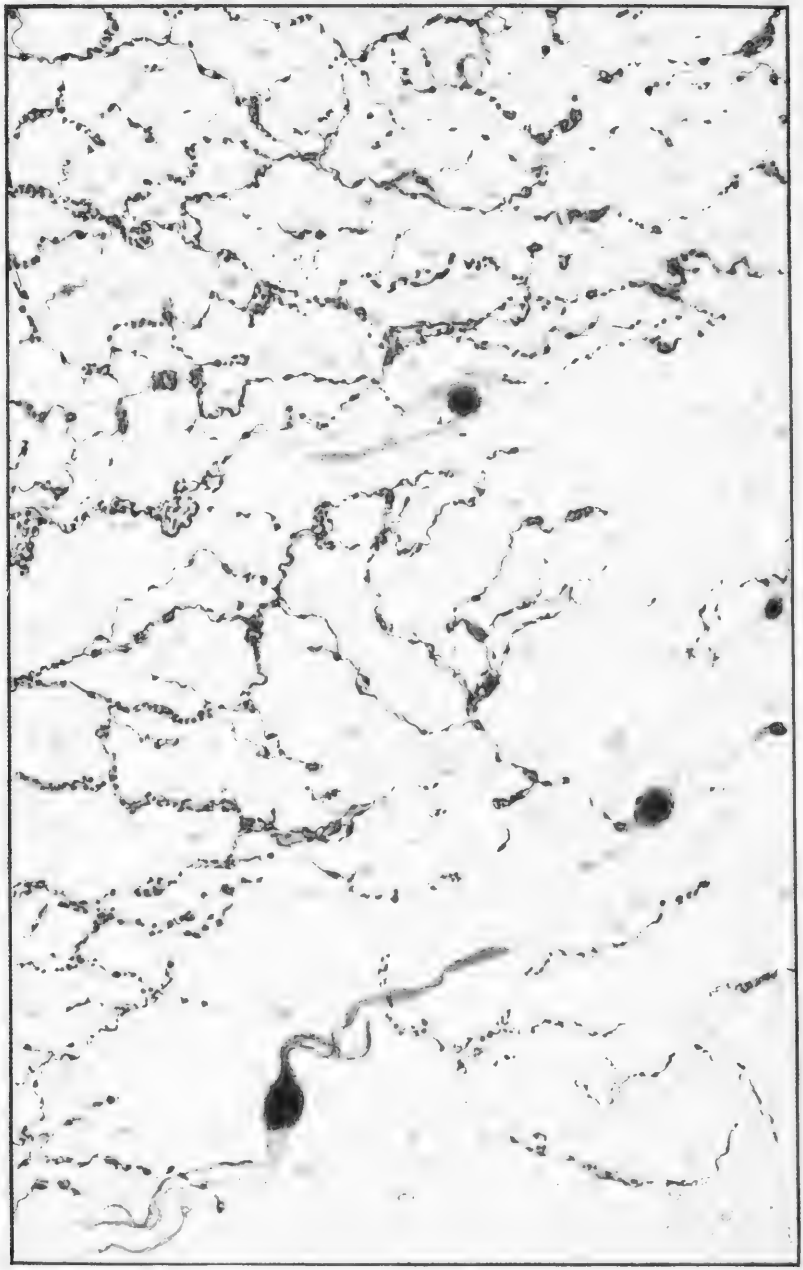


FIG. 1.

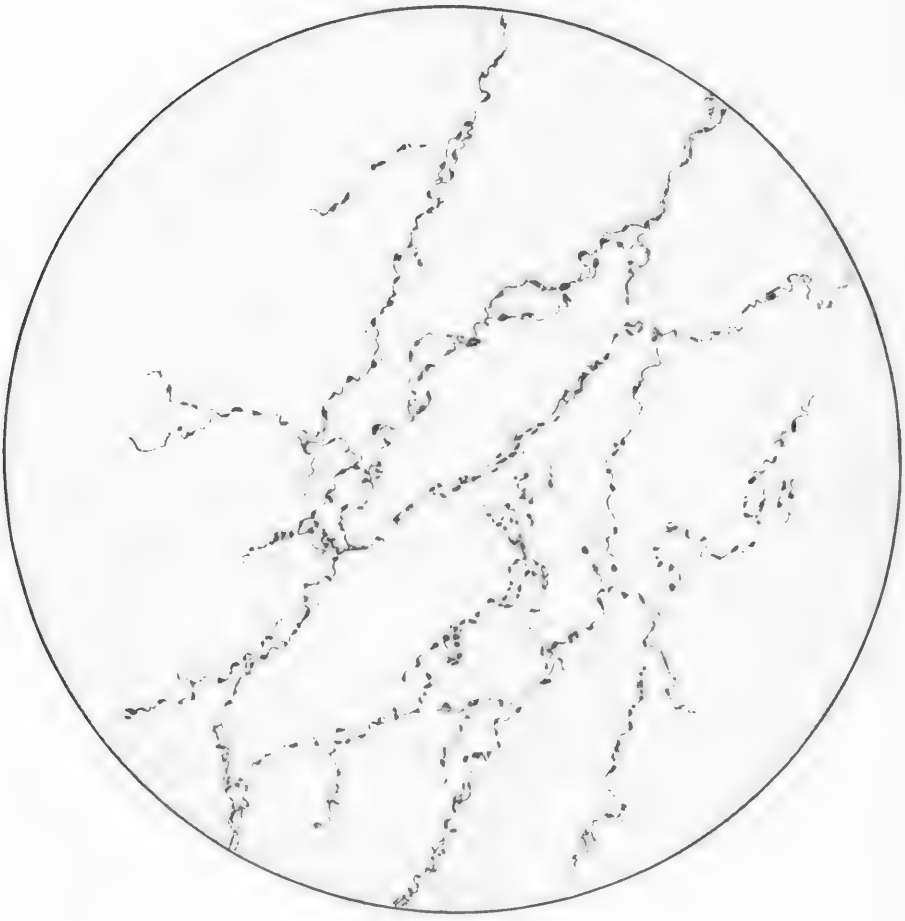


FIG. 2.

FIG 3.

EXPLANATION OF PLATES.

Plate 65.

Fig. 1. Plexus of varicose nerve fibrils in close relation to the characteristic muscle fibers of the sino-auricular node of the pig. A multipolar ganglion cell is shown at the left hand margin of the plate. Stained with methylene-blue and alum-carmine.

Plate 66.

Fig. 2. Plexus of moniliform nerve fibrils in close relation to the sino-auricular musculature of the bullock. Stained with methylene-blue.

Plate 67.

Fig. 3. A coarse varicose neurofibril in the sino-auricular node of the bullock, apparently breaking up into finer moniliform fibrils in close relation to the nodal muscle fibers.

CULTIVATION OF SPIROCHÆTA GALLINARUM.*

By HIDEYO NOGUCHI, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research, New York.*)

PLATE 68.

Spirochæta gallinarum, discovered in 1903 by Marchoux and Salimbeni[1] in an epidemic in Brazil, is a septicemic organism responsible for a febrile disease of the chicken which often terminates fatally. The infection is transmitted by certain species of ticks (*Argas miniatus, Argas persicus, Argas reflexus, Ornithodorus moubata*), and the symptoms appear usually within three or four days after the tick's bite, and last for several days; there is no relapse. At the height of the fever the blood swarms with the spirochætæ, but in the event of recovery, they disappear within several days. *Spirochæta gallinarum* bears resemblance to *Spirochæta anserina,* discovered by Sakharoff[2] in the blood of geese and a spirochæta found by Balfour[3] in a febrile chicken disease in Sudan, and Nuttall considers that the three forms are probably identical. According to Sakharoff, the anserina is incapable of infecting the chicken, while the gallinarum can infect not only the chicken, but sparrows, geese, and rabbits.

Spirochæta gallinarum bears a close morphological resemblance to spirochætæ causing relapsing fevers in man, but is somewhat more delicate than they. According to Novy and Knapp,[4] the gallinarum measures about 0.25 of a micron in width and from 10 to 12 microns in length. In a fresh preparation of blood from infected birds it exhibits remarkable motility characterized by a swift forward movement and a lateral vibration of the entire body. It often forms a ring, turning first in one direction, then in the reverse direction.

* Received for publication, August 15, 1912.

[1] Marchoux, E., and Salimbeni, A., *Ann. de l'Inst. Pasteur,* 1903, xvii, 569.

[2] Sakharoff, M. N., *Ann. de l'Inst. Pasteur,* 1891, v, 564.

[3] Balfour, *Reports of The Wellcome Research Laboratories,* 1908, iii, 38.

[4] Novy, F. G., and Knapp, R. E., *Jour. Infect. Dis.,* 1906, iii, 291.

In some specimens when one end of the body is attached to the slide a swinging movement may be observed.

Spirochæta gallinarum has not previously been successfully cultivated outside the body. Borrel and Burnet[5] observed an initial multiplication of the organism when a portion of the infected blood was mixed with chicken plasma, either pure or citrated, to which some bouillon was added afterwards; but no multiplication took place beyond the first tube. Levaditi[6] observed multiplication for many generations by cultivating the spirochætæ in the peritoneal cavity of rabbits in collodion sacs containing infected blood.

In the course of my studies on the cultivation of spirochætæ of relapsing fevers,[7] I undertook to cultivate *in vitro Spirochæta gallinarum,* and with success. I have now secured many generations by passing the culture from tube to tube at appropriate intervals.

Material.—Two different strains of *Spirochæta gallinarum* were employed. The first strain was kindly furnished by Professor Ehrlich in infected ticks from which the spirochæta was subsequently transferred to chickens. The infection of chickens by the ticks readily succeeded. The second strain of the gallinarum I owe to the courtesy of Dr. Levaditi of the Pasteur Institute of Paris. It was sent in infected blood hermetically sealed in glass tubes. Upon arrival, fifteen days later, a few motile spirochætæ were still seen under the dark-field microscope. The blood was first inoculated into canary birds and from the canary to the chicken.

For the purpose of cultivation the blood was aseptically taken under ether anesthesia from the heart of the infected birds and mixed immediately with a sufficient quantity of sodium citrate salt solution to prevent coagulation. The resulting emulsion of the blood which contained varying numbers of the spirochætæ was used for inoculating the culture media.

Culture Media.—The essential constituents of the culture media are: (1) fresh tissue, (2) ascitic fluid, and (3) paraffin oil. It is understood that the tissue, ascitic fluid, and paraffin oil must be absolutely sterile. The tissue must be perfectly fresh and of proper size.

[5] Borrel, A., and Burnet, E., *Compt. rend. Soc. de biol.*, 1906, lx, 540.
[6] Levaditi, C., *Compt. rend. Soc. de biol.*, 1906, lx, 688.
[7] Noguchi, H., *Jour. Exper. Med.*, 1912, xvi, 199.

Kidneys of normal rabbits or pectoral muscles of chicken are best suited for this purpose. The ascitic fluid must be tested for its suitability, as many specimens are wholly unsuitable. The selection is to be made by actual cultivation experiment, which is done with several specimens of ascitic fluids simultaneously. This may seem very tedious, but when one finds good specimens, they can be kept on ice for future work and there will be no difficulty in continuing the cultivation for any number of transfers. The paraffin oil is best sterilized twice in an autoclave.

Technique of Cultivation.—Cultivation of this organism is simple and consists in placing a piece of fresh sterile tissue (about the size of a chestnut) and ascitic fluid in a sterile test-tube, followed by the introduction of several drops of the blood emulsion containing the spirochætæ. A layer of sterile paraffin oil is now poured in. The amount of ascitic fluid in the culture tube should be sufficient to reach a height of about ten centimeters. Incubation is at 37° C.

Mindful of the frequent occurrence of bacterial contamination, it is my custom to inoculate more than one culture tube on each occasion in order to obtain at least one pure culture. Bacterial contamination is a serious obstacle in the work, as when it occurs, it cannot be removed without passing the culture once more into chickens; sometimes the growth of the culture may be entirely suppressed according to the nature of the contaminating organisms.

Properties of Pure Culture.—Spirochæta gallinarum increases considerably in number within twenty-four hours of inoculation into the culture media. The rate of multiplication continues to be very rapid during the following three days, at which time several spirochætæ are found in each microscopic field. On the fifth day the growth comes to a standstill, and on or after the sixth day degenerative processes, indicated by reduction or loss of motility of many organisms and the irregularity of their curves, commence to prevail. While the majority of the spirochætal organisms undergo disintegration within ten days, a few motile ones are still present in the cultures kept at 37° C. on the fifteenth day, and very rarely is a living specimen present in cultures older than three weeks.[8] The onset of

[8] An exception to this rule has recently been met with while examining some culture tubes which had not been disturbed since inoculation thirty days previously and which had been continuously kept in the thermostat at 37° C. There were still quite a few motile spirochætæ.

the degenerative phase takes place gradually and is in striking contrast to the suddenness with which it occurs in the cultures of *Spirochætæ duttoni, kochi, obermeieri,* and *novyi.*

The appearance of the culture media is but little modified by the growth of *Spirochæta gallinarum,* except that under certain conditions a very faint opalescence may appear. This happens especially when a piece of pectoral muscle of the chicken is used instead of rabbit kidney. No noticeable odor is produced in the culture.

No growth is obtained at room temperature or *in vacuo* at 37° C. Thus it is clear that the organism requires for its growth a certain amount of oxygen. In this respect the gallinarum resembles the spirochætæ of human relapsing fevers.

Subcultures.—Transplantation from culture to culture can be kept up for many generations and probably for indefinite periods, provided that a suitable specimen of ascitic fluid be employed. I have been able to continue cultivation of the two strains I worked with for fifteen successive transfers during the past two months. In continuous cultivation, the importance of suitable ascitic fluids cannot be too much emphasized.

Morphology.—(Figures 1 to 12.) In culture media containing a piece of fresh muscle of chicken and ascitic fluid, *Spirochæta gallinarum* presents all the morphological characteristics observed in the blood of an infected chicken. The organism measures about 0.3 of a micron in width and from 8 to 16 microns in length. Cultures three or four days old show numerous chains of two, three, or even four organisms. The organisms may be joined together by a short, thin filament that measures less than two microns in length, but sometimes the joint is merely suggested from the absence of curves, and there is no actual thinning. The number of curves varies according to the length of the specimen, but the distance between the curves is constant, measuring about 1.8 microns. Average curves have a depth of about one micron and usually a round apex. The position of the curves appears to change especially when the spirochætæ manifest violent movements. The regularity of the curves is most marked in the specimens which show moderate motility.[9] The

[9] The regularity of curves may be more or less lost during the process of drying on a slide, so that the stained preparation shows rather irregularly curved spirochætæ (figure 12).

movements are chiefly rotatory with more or less marked lateral vibration. The forward locomotion is less noticeable in the culture than in the blood. Agglomeration of spirochætæ is seldom observed even at the height of growth (usually on the fifth day) and in this respect the condition of culture differs from that of the blood of an infected bird.

Spirochæta gallinarum usually possesses a delicate, finely curved projection attached to one end of the body, measuring about three microns. It is rare to find a specimen with similar projections at both ends. In some specimens the projection is devoid of curves and appears to be more fragile than the curved ones. I am of the opinion that the straight projection is a prolongation of periblast and the curved one is continuous with the axial filament of the spirochæta. I arrived at this conclusion from my observations on cultured *Treponemata* (including the *pallidum, microdentium, macrodentium, mucosum, refringens,* and other relapsing fever spirochætæ).

Under somewhat less favorable cultural conditions certain atypical forms arise. These organisms may be short, having only two or three curves, or the curves may be irregular. This irregularity may also arise when a less suitable ascitic fluid is used and in a culture entering on the degenerative phase.

In all cultures one invariably finds varying numbers of round refractory bodies that are attached to the periblast of the spirochætæ. As a rule, one organism carries one such body which may be attached to a point anywhere along the side of the spirochæta. This spherical body measures about 0.75 of a micron. The number of the organisms with round bodies is inconstant in different cultures, but it seems to become greater where the growth of the culture approaches its maximum. Usually it is absent in cultures not older than two days. Apparently not every culture is capable of producing these bodies. Similar round bodies have been observed by previous investigators not only in the blood of the infected birds, but also in the fresh preparations of various other spirochætæ. I have described the presence of such round bodies in pure cultures of *Treponema pallidum, Treponema microdentium, Treponema macrodentium, Treponema mucosum,*[10] *Spirochæta refringens, Spiro-*

[10] Noguchi, H., *Jour. Exper. Med.,* 1912, xvi, 194.

chæta phagedenis,[11] *Spirochæta duttoni, Spirochæta kochi, Spirochæta obermeieri,* and *Spirochæta novyi.* The conditions under which the bodies are formed suggest that they represent one of the phases of the life cycle and should not be viewed as the result of plasmolysis, as some investigators have assumed.[12] At the same time they are certainly not the encystment forms described by Perrin,[13] von Prowazek,[14] and others in certain spirochætæ.

Another phenomenon observed in pure cultures of *Spirochæta gallinarum,* although common with all other spirochætæ studied by me in culture, is the formation of numerous granules in certain cultures, especially when their maximum growth has passed or the media have been somewhat defective. These granules are round or ovoid and are highly refractory. They do not exceed 0.3 of a micron and usually are about 0.2 of a micron in diameter. They show active molecular movements. They have doubtless been derived from the spirochætæ, since they are attached to numerous organisms along the entire body length. These granular spirochætæ may be quite actively motile or may show no sign of life. When the cultures are examined carefully, the granular spirochætæ are observed to be actually undergoing degeneration. On examinations day by day, these spirochætæ are seen gradually to give up the granules until they become completely or almost completely denuded, their immobile skeletal axial filaments alone remaining. In my opinion, the entire process is one of the degeneration, and the granules are nothing but the fragments of the periblast of the spirochætæ. Whether or not this phenomenon has any relation to the formation of coccoid bodies described by Balfour,[15] Leishman,[16] Bosanquet,[17] Hindle,[18] and others in blood specimens, I cannot say. At all events, the granules just described in cultures are incapable of

[11] Noguchi, H., *Jour. Exper. Med.,* 1912, xvi, 261.
[12] Hindle, E., *Parasitology,* 1912, iv, 463.
[13] Perrin, W. S., *Arch. f. Protistenk.,* 1906, xii, 131.
[14] von Prowazek, S., *Arb. a. d. k. Gsndhtsamte,* 1906, xxiii, 554; *Mem. do Inst. Oswaldo Cruz,* 1909, i, 79.
[15] Balfour, A., *Jour. Trop. Med.,* 1911, xiv, 113.
[16] Leishman, W. B., *Lancet,* 1910, i, 11.
[17] Bosanquet, W. C., *Quart. Jour. Micr. Sc.,* 1911, lvi, 387.
[18] Hindle, E., *loc. cit.*

causing the infection in chickens or of being cultivated in the media described.

While the morphological features of *Spirochæta gallinarum* in culture media containing chicken tissue remain typical for many generations, the organisms cultivated in media with rabbit tissue become somewhat thicker and the curves appear shallower. By transplanting these organisms back to the chicken media they once more become typical. On the other hand, the spirochæta grows just as abundantly in the presence of rabbit tissue as it does in the presence of chicken muscle.

In regard to the mode of multiplication I was able to observe unmistakable instances of transverse division by strangulation, but no longitudinal division could be detected. Whether or not the latter mode of division does not occur also under certain conditions must be determined later.

At no period of cultivation did bacillary forms appear, such as are described by Hindle[19] as one of the phases of the life cycle of this organism in the tick.

Filterability.—In view of the findings of Novy and Knapp,[20] who caused an infection by injecting the filtrate of the blood derived from animals infected with *Spirochæta novyi,* I made several series of experiments in order to determine whether the cultures of *Spirochæta gallinarum* contained at any period a filterable form. For this purpose a mixture of cultures of varying ages (from two to thirty days) was filtered through several Berkefeld filters (V). The filtrate was tested in two ways. It was inoculated into chickens and cultured in suitable media. Controls for both series were provided with unfiltered cultures. Neither infection of chickens nor culture was obtained with the filtrates. In this connection I may mention that similar experiments performed with cultures of *Spirochæta duttoni, kochi, obermeieri,* and *novyi* gave negative results. These experiments do not affect the positive results obtained by Novy and Knapp, as they used filters which had been ground to lessen the thickness of the wall.

Pathogenicity.—*Spirochæta gallinarum* in culture does not lose

[19] Hindle, E., *loc. cit.*

[20] Novy, F. G., and Knapp, R. E., *loc. cit.*

its virulence for the chicken to any noticeable degree when the transplantations are kept up at regular intervals in a suitable medium. Thus the cultures after thirteen transplantations caused fatal infection in chickens with characteristic symptoms of the disease. It remains to be determined by future experiments whether or not prolonged cultivation in artificial media will finally bring about an attenuation or abolition of virulence of this organism. I have found incidentally that when the transplantations into new media are neglected for about four weeks a culture which still shows numerous motile organisms no longer causes the infection in the chicken, even where a large quantity of the culture is inoculated.[21] This seems to indicate that the gallinarum may become attenuated in virulence under certain cultural conditions; perhaps the attenuated cultures may confer immunity.

CONCLUSIONS.

1. *Spirochæta gallinarum* can be cultivated in suitable artificial media for many successive generations and probably for indefinite periods. The presence of fresh tissue and a certain amount of oxygen seems to be essential for its growth. No perceptible odor is produced in the cultures.

2. The maximum growth of *Spirochæta gallinarum* is reached on about the fifth day, but the phase of degeneration commences slowly and gradually, so that in this respect the gallinarum differs from the duttoni, kochi, obermeieri, or novyi, whose cultures are characterized by sudden onset of degeneration soon after the maximum growth is attained.

3. No rod formation resembling bacilli arises in the course of multiplication of *Spirochæta gallinarum* in cultures. Many round or oval bodies appear in old cultures, but no infection of animals or formation of spiral forms from these granules has been produced. The granules are probably the degeneration products derived from the periblast of the spirochætæ.

4. Cultures of *Spirochæta gallinarum,* either old or young, do

[21] The chickens which received the inoculation of the attenuated culture resisted the infection against a virulent strain directly from an infected bird when tested two weeks afterwards.

not contain a form which passes through a Berkefeld filter (V) that infects chickens or grows into spirochætæ.

5. *Spirochæta gallinarum* remains virulent for chickens after being in cultures for at least thirteen generations, but it may become avirulent under certain cultural conditions. The inoculation of chickens with the attenuated culture renders the birds refractory to the subsequent infection with a virulent strain.

6. When the spirochætæ are cultivated in the media containing rabbit kidney instead of chicken muscle, the individual specimens are somewhat thicker, but otherwise typical.

7. *Spirochæta gallinarum* multiplies in culture by transverse division. No positive evidence of a longitudinal division has been obtained.

EXPLANATION OF PLATE 68.

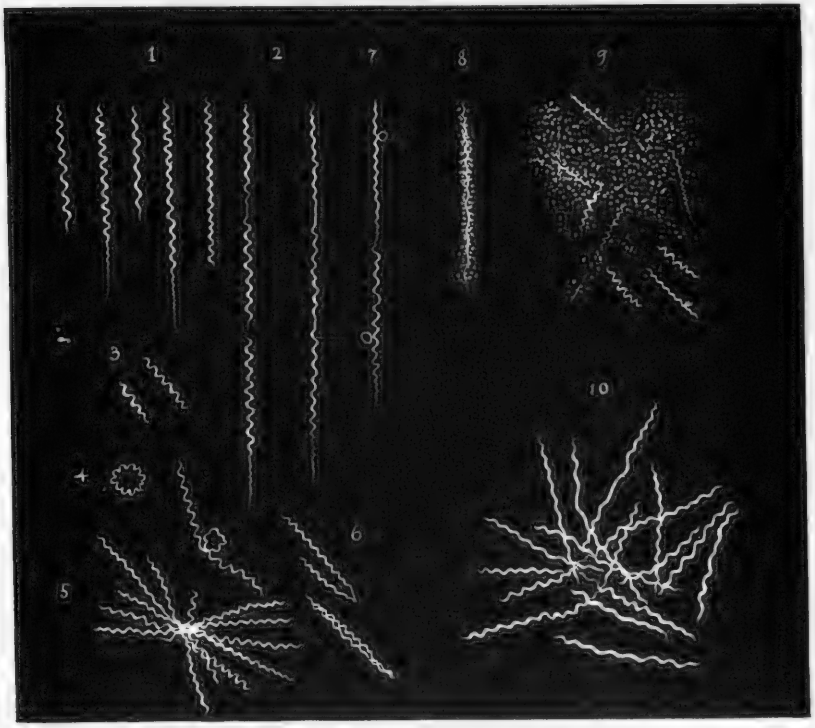
FIGS. 1 to 10. Schematic reproductions of *Spirochæta gallinarum* in pure culture, as observed under the dark-field microscope. Different stages of growth of the spirochætæ in a medium containing ascitic fluid and chicken muscle.

FIG. 1. Average forms; FIG. 2, long chains; FIG. 3, short forms; FIG. 4, ring formation; FIG. 5, agglomeration and knot formation; FIG. 6, doubled up forms; FIG. 7, spirochætæ with spore-like spherical bodies; FIG. 8, a spirochæta with granules; FIG. 9, degeneration phase with numerous granules, skeletal axial filaments, and several short motile spirochætæ.

FIG. 10. *Spirochæta gallinarum* in a culture medium containing ascitic fluid and rabbit kidney, showing somewhat thicker and less regularly curved forms.

FIG. 11. *Spirochæta gallinarum* from a pure culture (20th generation). × 1,100. Dark-field view.

FIG. 12. *Spirochæta gallinarum* from a pure culture (20th generation) fixed in methyl alcohol and stained with the Giemsa solution for four hours. × 1,100.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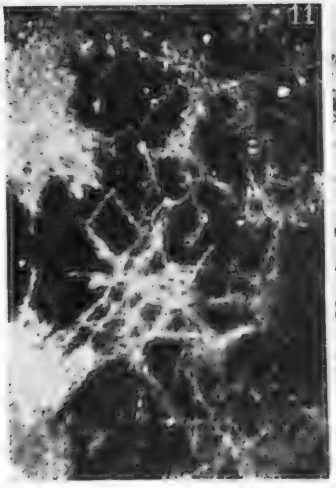
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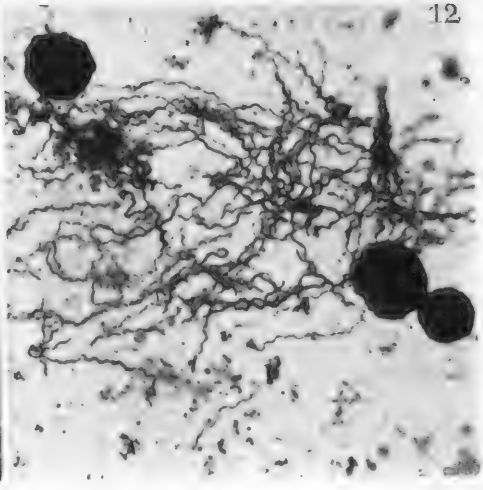

12

THE DURATION AND EXTENT OF INDUCED RESISTANCE TOWARD TUMOR TRANSPLANTATION IN MICE.*

By WILLIAM H. WOGLOM, M.D.

(*From the Laboratories of The Imperial Cancer Research Fund, London.*)

The extent and duration of the resistance which various tissues are able to evolve against the implantation of tumors has not yet been systematically examined and compared. The most complete experiments so far recorded in the literature are those of Ehrlich[1] which, however, concern themselves only with the immunity produced by tumors. Resistance was discovered from seven to fourteen days after the unsuccessful inoculation of spontaneous growths, and persisted for weeks or even months, while the immunity produced by transplantable tumors was distinct at the ninth day, and remained high for at least three months.

Bashford,[2] in discussing the resistance present after spontaneous absorption of Jensen's mouse tumor, said that it continued for at least six months.

As for the rat, Uhlenhuth, Haendel, and Steffenhagen[3] found that resistance was present for at least six weeks after the absorption of a spindle cell sarcoma, while Flexner and Jobling[4] estimated that the refractory condition initiated by the absorption of an adenocarcinoma was reduced by the mere lapse of time, but persisted for at least ninety days.

* The expenses of this investigation were borne in part by the George Crocker Special Research Fund. Received for publication, July 3, 1912.

[1] Ehrlich, P., *Ztschr. f. ärztl. Fortbild.*, 1906, iii, 211; *Arb. a. d. k. Inst. f. exper. Therap. zu Frankfurt*, 1906, No. 1, 90, 97.

[2] Bashford, E. F., *Brit. Med. Jour.*, 1906, ii, 209.

[3] Uhlenhuth, Haendel, and Steffenhagen, *Centralbl. f. Bakteriol., Ref., 1te Abt.*, 1910, xlvii, Suppl., 164.

[4] Flexner, S., and Jobling, J. W., *Monographs of The Rockefeller Institute for Medical Research*, 1910, No. 1, 54.

Bridré[5] investigated the resistance produced in mice by unsuccessful tumor inoculation, as well as that entailed by treatment with normal tissue, and concluded that it might endure for five months or more. That attendant upon the inoculation of mouse blood appeared after four days, according to Bashford, Murray, and Cramer,[6] but was more distinct after ten days, and continued for at least three weeks. In the experiments of Russell,[7] embryo mouse skin required twenty days to elicit the full refractory condition.

The occurrence of spontaneous tumors in mice which have become immune through unsuccessful tumor inoculation, as reported by Bashford, Murray, and Cramer,[8] Thorel,[9] and Clunet,[10] possesses a certain subsidiary interest in its relations to the duration of immunity. The sporadic growths recorded by these investigators occurred from ten days to nine months after immunization, but as the average duration of induced resistance is only three months, according to the authors previously cited, it may be questioned whether the refractory condition was still in existence in those mice which developed spontaneous tumors more than that length of time after immunization. Since, however, the conditions essential for the inception of malignant growth are in all probability distinct from those necessary for its continuation, as Bashford has repeatedly emphasized, the question whether or not tumors have ever arisen in artificially immunized mice can hardly be of more than academic interest, except in so far as it touches the hypothesis which would ascribe cancer to the invasion and growth of an exogenous cell.

[5] Bridré, J., *Ann. de l'Inst. Pasteur,* 1907, xxi, 773.

[6] Bashford, E. F., Murray, J. A., and Cramer, W., *Proc. Roy. Soc., Series B,* 1907, lxxix, 180; *Third Scientific Report of the Imperial Cancer Research Fund,* 1908, 333.

[7] Russell, B. R. G., *Third Scientific Report of the Imperial Cancer Research Fund,* 1908, 345.

[8] Bashford, E. F., Murray, J. A., and Cramer, W., *Third Scientific Report of the Imperial Cancer Research Fund,* 1908, 171, 322; Bashford, E. F., Murray, J. A., and Haaland, M., *ibid.,* 396.

[9] Thorel, *Verhandl. d. deutsch. path. Gesellsch.,* 1908, xii, 60.

[10] Clunet, J., Recherches expérimentelles sur les tumeurs malignes, Paris, 1910, 24.

EXPERIMENTAL PART.

The experiments about to be described are directed toward a contrast of the immunizing power of propagated growths and spontaneous tumors, all adenocarcinomata of the mouse and most of them hemorrhagic, with that of normal tissue, the latter represented by kidney or embryo skin. The immunizing dose of tumor, kidney, or embryo skin, except in the case of transplantable carcinoma 206, where 0.03 of a cubic centimeter was given, was 0.05 of a cubic centimeter of an emulsion, which was deposited in the left axilla by means of a syringe. The testing inoculation of tumor took place in the right axilla after an appropriate interval had elapsed, there being employed for this purpose carcinomata T and 91, each significant for its large percentage of takes, its uniform and progressive growth, and its inability to induce concomitant immunization. The inoculation yield with carcinoma T was estimated twenty-four days after inoculation, but with carcinoma 91 seventeen days, because of the more rapid growth of this tumor. On account of the proliferative activity of these two growths, it rarely happens that by the second or third charting the nodules are still so small as to render difficult an estimation of the result, and less than a dozen animals, therefore, among the sixteen hundred odd comprised in the experiment, had to be discarded because the outcome was doubtful. The small number of mice which died before the final charting were included if death had been postponed long enough to allow a definite decision to be reached. Otherwise they were eliminated from the experiment, as were those few in which the spontaneous growth employed for immunization had succeeded in gaining a foothold. All the mice used for controls were of the same age as the immunized animals, and were in every instance taken from the same batch and kept under identical conditions until the time for inoculation arrived.

The curves which follow show the degree of immunity as it was revealed at stated intervals. The ratio between the negative mice and the total number of mice in each group, expressed as a percentage, is taken as representing the extent of resistance, and the curves are, therefore, the obverse of those usually published to set forth the proportion of successful inoculations. The details upon which

they are founded are appended in a series of tables numbered to correspond with the charts (tables I to VI).

TABLE I.

Immunizing material.	Interval.	Testing inoculation.	Proportion of negatives.	Per cent. of immunity.
Embryo skin 0.05 c.c.	5 days.	*T*/36*B* 0.01 c.c.	8/13	61
Tumor 614 0.05 c.c.	5 days.	*T*/36*B* 0.01 c.c.	1/13	8
Control (untreated)		*T*/36*B* 0.01 c.c.	2/16	12
Embryo skin 0.05 c.c.	10 days.	*T*/34*I* 0.01 c.c.	17/17	100
Tumor 541 0.05 c.c.	10 days.	*T*/34*I* 0.01 c.c.	7/9	78
Control (untreated)		*T*/34*I* 0.01 c.c.	2/15	13
Embryo skin 0.05 c.c.	17 days.	*T*/35*A* 0.01 c.c.	16/17	94
Tumor 556 0.05 c.c.	17 days.	*T*/35*A* 0.01 c.c.	7/23	30
Control (untreated)		*T*/35*A* 0.01 c.c.	3/15	20
Embryo skin 0.05 c.c.	21 days.	*T*/35*C* 0.01 c.c.	18/19	95
Tumor 555 0.05 c.c.	21 days.	*T*/35*C* 0.01 c.c.	3/14	21
Control (untreated)		*T*/35*C* 0.01 c.c.	3/17	18
Embryo skin 0.05 c.c.	24 days.	*T*/34*G* 0.01 c.c.	19/19	100
Tumor 534 0.05 c.c.	24 days.	*T*/34*G* 0.01 c.c.	3/18	17
Control (untreated)		*T*/34*G* 0.01 c.c.	4/16	25
Embryo skin 0.05 c.c.	31 days.	*T*/34*H* 0.01 c.c.	5/11	45
Tumor 534 0.05 c.c.	31 days.	*T*/34*H* 0.01 c.c.	2/17	12
Control (untreated)		*T*/34*H* 0.01 c.c.	1/15	7
Embryo skin 0.05 c.c.	38 days.	*T*/34*J* 0.01 c.c.	13/17	76
Tumor 540 0.05 c.c.	38 days.	*T*/34*J* 0.01 c.c.	3/17	18
Control (untreated)		*T*/34*J* 0.01 c.c.	0/14	0
Embryo skin 0.05 c.c.	50 days.	*T*/41*D* 0.01 c.c.	15/24	62
Tumor 635 0.05 c.c.	50 days.	*T*/41*D* 0.01 c.c.	10/27	37
Control (untreated)		*T*/41*D* 0.01 c.c.	4/24	17
Embryo skin 0.05 c.c.	75 days.	*T*/44*E* 0.01 c.c.	7/28	25
Tumor 687 0.05 c.c.	75 days.	*T*/44*E* 0.01 c.c.	4/23	17
Control (untreated)		*T*/44*E* 0.01 c.c.	0/21	0

Although the configuration of the curves is of interest, showing as it does the rise and fall of resistance, these do not lend themselves for the exact mathematical analysis to which Ehrlich, and Arrhenius and Madsen have subjected their own curves illustrating the course of immunity in general. This is because, in the first place, the outcome of tumor inoculation, even when it is undertaken in normal untreated animals, is variable. Furthermore, it is not possible to read the result immediately, as it is in experiments which concern themselves with hemolysins, precipitins, or other bodies in-

TABLE II.

Immunizing material.	Interval.	Testing inoculation.	Proportion of negatives.	Per cent. of immunity.
Embryo skin 0.05 c.c.	5 days.	*T*/38*C* 0.01 c.c.	10/16	62
Tumor 665 0.05 c.c.	5 days.	*T*/38*C* 0.01 c.c.	14/20	70
Control (untreated)		*T*/38*C* 0.01 c.c.	3/18	17
Embryo skin 0.05 c.c.	10 days.	*T*/36*A* 0.01 c.c.	11/12	92
Tumor 550 0.05 c.c.	10 days.	*T*/36*A* 0.01 c.c.	17/18	94
Control (untreated)		*T*/36*A* 0.01 c.c.	1/14	7
Embryo skin 0.05 c.c.	17 days.	*T*/36*D* 0.01 c.c.	20/24	83
Tumor 587 0.05 c.c.	17 days.	*T*/36*D* 0.01 c.c.	6/20	30
Control (untreated)		*T*/36*D* 0.01 c.c.	0/12	0
Embryo skin 0.05 c.c.	21 days.	*T*/35*D* 0.01 c.c.	9/12	75
Tumor 533 0.05 c.c.	21 days.	*T*/35*D* 0.01 c.c.	4/9	44
Control (untreated)		*T*/35*D* 0.01 c.c.	0/8	0
Embryo skin 0.05 c.c.	24 days.	*T*/39*B* 0.01 c.c.	16/19	84
Tumor 521 0.05 c.c.	24 days.	*T*/39*B* 0.01 c.c.	8/20	40
Control (untreated)		*T*/39*B* 0.01 c.c.	2/22	9
Embryo skin 0.05 c.c.	31 days.	*T*/36*B* 0.01 c.c.	10/13	77
Tumor 541 0.05 c.c.	31 days.	*T*/36*B* 0.01 c.c.	6/11	54
Control (untreated)		*T*/36*B* 0.01 c.c.	3/11	27
Embryo skin 0.05 c.c.	38 days.	*T*/37*B* 0.01 c.c.	7/9	78
Tumor 549 0.05 c.c.	38 days.	*T*/37*B* 0.01 c.c.	2/14	14
Control (untreated)		*T*/37*B* 0.01 c.c.	2/17	12
Embryo skin 0.05 c.c.	50 days.	*T*/43*Q* 0.01 c.c.	10/17	59
Tumor 711 0.05 c.c.	50 days.	*T*/43*Q* 0.01 c.c.	7/23	30
Control (untreated)		*T*/43*Q* 0.01 c.c.	0/18	0
Embryo skin 0.05 c.c.	75 days.	*T*/45*A* 0.01 c.c.	15/27	55
Tumor 697 0.05 c.c.	75 days.	*T*/45*A* 0.01 c.c.	4/14	28
Control (untreated)		*T*/45*A* 0.01 c.c.	7/30	23

TABLE III.

Immunizing material.	Interval.	Testing inoculation.	Proportion of negatives.	Per cent. of immunity.
Embryo skin 0.05 c.c.	3 days.	91/37*B* 0.02 c.c.	4/23	17
Control (untreated)		91/37*B* 0.02 c.c.	0/19	0
Embryo skin 0.05 c.c.	5 days.	91/37*D* 0.02 c.c.	14/20	70
Control (untreated)		91/37*D* 0.02 c.c.	0/18	0
Embryo skin 0.05 c.c.	10 days.	91/37*C* 0.02 c.c.	18/23	78
Control (untreated)		91/37*C* 0.02 c.c.	0/24	0
Embryo skin 0.05 c.c.	17 days.	91/37*G* 0.02 c.c.	22/23	96
Control (untreated)		91/37*G* 0.02 c.c.	1/25	4

TABLE IV.

Immunizing material.	Interval.	Testing inoculation.	Proportion of negatives.	Per cent. of immunity.
Embryo skin 0.05 c.c.	3 days.	91/38*D* 0.02 c.c.	4/18	22
Control (untreated).....		91/38*D* 0.02 c.c.	0/18	0
Embryo skin 0.05 c.c.	5 days.	91/38*F* 0.02 c.c.	10/17	59
Control (untreated).....		91/38*F* 0.02 c.c.	2/16	12
Embryo skin 0.05 c.c.	10 days.	91/37*F* 0.02 c.c.	19/23	83
Control (untreated).....		91/37*F* 0.02 c.c.	3/22	14
Embryo skin 0.05 c.c.	17 days.	91/38*C* 0.02 c.c.	12/14	86
Control (untreated).....		91/38*C* 0.02 c.c.	2/19	10

TABLE VI

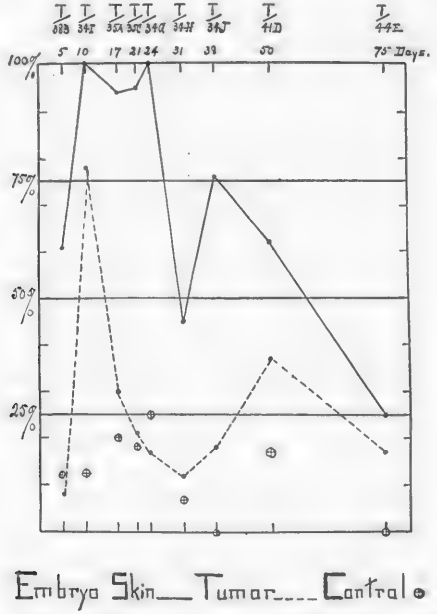
Immunizing material.	Interval.	Testing inoculation.	Proportion of negatives.	Per cent. of immunity.
Kidney 0.05 c.c.....	5 days.	*T*/41*F* 0.01 c.c.	5/15	33
Control (untreated).....		*T*/41*F* 0.01 c.c.	1/20	5
Kidney 0.05 c.c.....	10 days.	*T*/41*G* 0.01 c.c.	14/21	67
Control (untreated).....		*T*/41*G* 0.01 c.c.	2/24	8
Kidney 0.05 c.c.....	17 days.	*T*/40*A* 0.01 c.c.	7/10	70
Control (untreated).....		*T*/40*A* 0.01 c.c.	0/12	0
Kidney 0.05 c.c.....	21 days.	*T*/42*A* 0.01 c.c.	9/21	43
Control (untreated).....		*T*/42*A* 0.01 c.c.	3/21	14
Kidney 0.05 c.c.....	24 days.	*T*/42*D* 0.01 c.c.	10/21	48
Control (untreated).....		*T*/42*D* 0.01 c.c.	2/18	11
Kidney 0.05 c.c.....	31 days.	*T*/43*O* 0.01 c.c.	10/20	50
Control (untreated).....		*T*/43*O* 0.01 c.c.	3/24	12
Kidney 0.05 c.c.....	38 days.	*T*/42*H* 0.01 c.c.	7/24	29
Control (untreated).....		*T*/42*H* 0.01 c.c.	2/18	11
Kidney 0.05 c.c.....	50 days.	*T*/43*G* 0.01 c.c.	11/35	31
Control (untreated).....		*T*/43*G* 0.01 c.c.	5/31	16
Kidney 0.05 c.c.....	75 days.	*T*/43*I* 0.01 c.c.	6/15	40
Control (untreated).....		*T*/43*I* 0.01 c.c.	3/20	15

TABLE VII

Immunizing material.	Interval.	Testing inoculation.	Proportion of negatives.		Per cent. of immunity.	
Tumor 206 0.03 c.c.	12 and 80 days.	63/56*D* 0.02 c.c. and *T*/43*L* 0.01 c.c.	11/11	3/11	100	27
Control (untreated).....		63/56*D* 0.02 c.c. and *T*/43*L* 0.01 c.c.	1/11	2/12	9	17

cluded in the province of the immunologist. On the contrary, there is interpolated between the testing inoculation and the ultimate estimation of the amount of resistance the length of time which is necessary for the tumors to obtain measurable proportions.

Text-figure 1 represents the onset and diminution of the immunity following the injection of embryo skin or spontaneous tumor. Even after five days there is resistance in something over



TEXT-FIG. 1. Curves showing the extent and duration of the resistance induced in mice with embryo mouse skin and spontaneous adenocarcinomata of the mammary gland of the mouse, respectively.

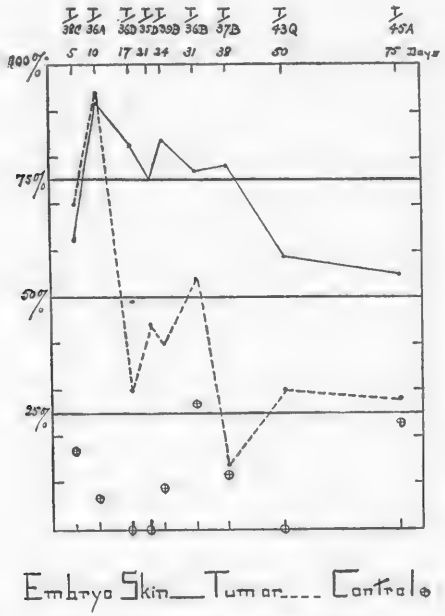
60 per cent. of the mice treated with embryo skin, while by the tenth day this immunity has risen to 100 per cent. Remaining at a high level until the twenty-fourth day, it then falls, until, at the seventy-fifth, it has reached 25 per cent., where it may be said practically to have vanished, since it falls within the resistance displayed by the control animals. The sharp drop at the thirty-first day is probably inside that margin of error for which allowance must be made in

biological experiments. By contrasting with this curve that which reproduces the immunity evolved by spontaneous tumors, it will be appreciated that the latter does not reach the high level attained by the former, and that it drops more rapidly. Why this should be so with spontaneous tumors, and, as will be seen later, also in the case of the kidney, is not clear. To ascribe it to the more rapid absorption of these two tissues in contrast to that obtaining with embryo skin, would be to refer immunity to the absorption of tissue. How unwarrantable this assumption may be, however, is suggested by the fact that animals with growing tumors in large part necrotic are not necessarily rendered resistant, although absorption of tissue may perfectly well be postulated; and that the failure of the absorbed material to produce immunity in such mice is not to be explained by the presence of a malignant growth is shown by the recent work of Russell,[11] who succeeded in conferring resistance even in the presence of a progressively growing transplanted tumor. For the present, then, one must be content merely to state that the resistance aroused by spontaneous tumor and by kidney is neither so intense nor of such long duration as that following treatment with embryo skin. The fact that the curve descends to a point where it is below the immunity exhibited by some of the controls, and rises again after having reached its lowest limit at the thirty-first day, is to be referred to experimental error. It is probable that under ideal conditions the immunity would disappear gradually, reaching 25 per cent. at about the fiftieth day.

Text-figure 2, depicting the outcome which ensued upon a duplication of the experiment under exactly parallel conditions, shows again the smaller degree of immunity evolved with spontaneous tumors as contrasted with embryo skin. The curve representing the immunizing power of embryo skin is, on the whole, a more level one, but suggests that more than seventy-five days may be required for the complete disappearance of the resistance following preliminary treatment with this material. Nevertheless, with an animal which, like the mouse, lives but a trifle more than two years, it would be injudicious to attempt an extension of the time element in

[11] Russell, B. R. G., *Fifth Scientific Report of the Imperial Cancer Research Fund*, 1912, 1.

such experiments as these, because mice which are immunized when they are about two months old have, at the expiration of seventy-five days, reached four months and a half, an age at which the comparative resistance displayed by older animals is likely to become a disturbing factor.

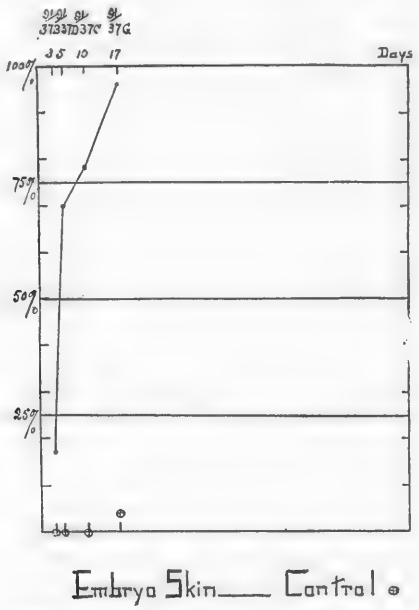


TEXT-FIG. 2. Curves showing the extent and duration of the resistance induced in mice with embryo mouse skin and spontaneous adenocarcinomata of the mammary gland of the mouse, respectively.

Because the rapid rise of the curve on these two charts might have been a result of the rather slow growth of adenocarcinoma T, and might not, therefore, correspond exactly with the rate at which resistance would develop against a tumor capable of establishing itself with more celerity, the first part of the experiment was twice repeated with adenocarcinoma 91, a neoplasm of such rapid proliferative energy as to yield within two weeks an amount of tissue varying between two and three grams, and this from an initial dose

of 0.02 of a cubic centimeter of emulsion. However, as text-figures 3 and 4 demonstrate, the curve is almost equally abrupt, and about 80 per cent. of immunity is obtained by the tenth day.

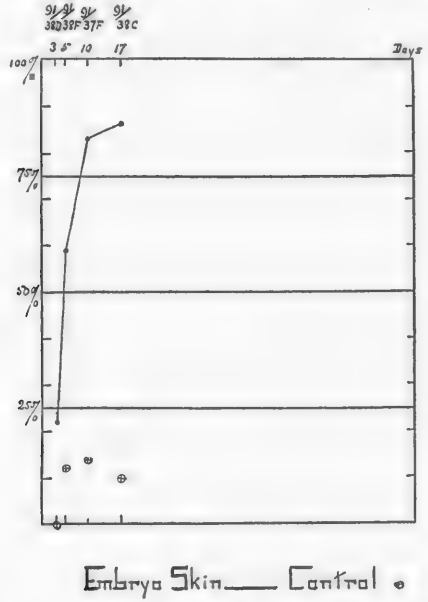
The salient feature in these two latter curves is the very rapid rise between the third and fifth days, a demonstration that within a period of but forty-eight hours the resistance has jumped from



TEXT-FIG. 3. Curve showing the abrupt rise of the resistance induced by embryo skin.

about 20 per cent. to 60 or 70 per cent. For this occurrence there are two possible explanations. As has been already pointed out, it is not possible to measure the amount of immunity actually present at the moment when the tumor was inoculated. Now, while the curves show that there is from 60 to 70 per cent. of resistance by the fifth day, this cannot be taken to mean that each of the mice in the experiment was from 60 to 70 per cent. immune, for it demonstrates, on the contrary, merely that six or seven mice out of ten

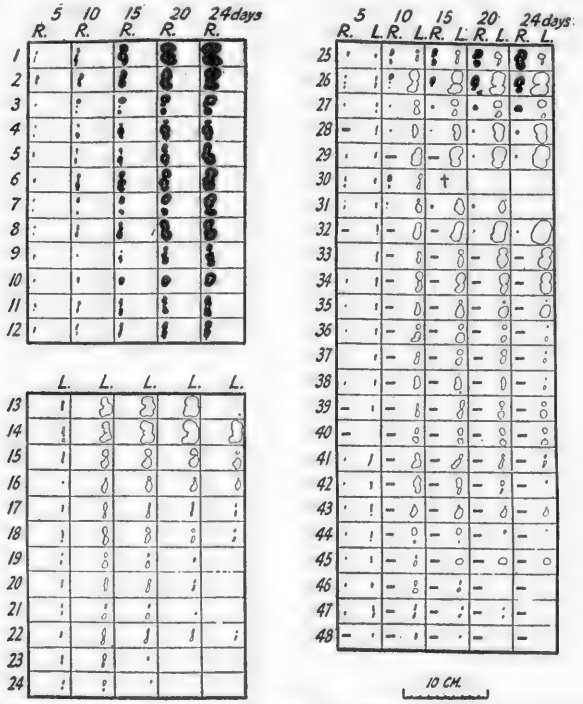
were immune. The two explanations turn upon the question whether the animals were partially or wholly immune. The first would assume that immunity was complete in 60 or 70 per cent. of the animals, and would state that this number of mice had become so resistant within five days after treatment as never to allow the tumor to gain a foothold at all. The second explanation, on the other hand, would suggest that the negative mice had not been



TEXT-FIG. 4. Curve showing the abrupt rise of the resistance induced by embryo skin.

absolutely resistant at the time of inoculation, but that a part of the refractory condition with which they were endowed had been conferred in the few days following the testing inoculation of tumor, to become operative against the graft several days after its introduction. As is well known, malignant cells upon their introduction into a new host force the tissues to provide them with a connective tissue scaffolding as well as a capillary blood supply, and this within

the first two or three days supervening upon their inoculation. The explanation now under discussion requires for its support, therefore, the subsidiary assumption that induced immunity may be valid even against a graft which is partially, or perhaps completely, vascu-

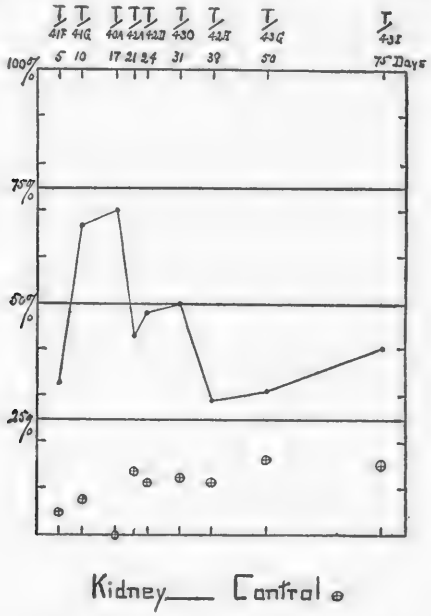


TEXT-FIG. 5. One tumor may immunize against a second, even though the two be implanted at the same time. Control mice 1 to 12 were inoculated with 0.01 c.c. of carcinoma 63, a tumor which does not immunize the host during its growth, while control mice 13 to 24 were injected with 0.01 c.c. of sarcoma 37, which does produce concomitant immunization. Mice 25 to 48 were inoculated simultaneously in the right and left axillæ with 0.01 c.c. of carcinoma 63 and sarcoma 37, respectively. Tumor 37 has been able to prevent the growth of 63.

Carcinoma 63 = black silhouette; sarcoma 37 = outline.

larized. That such a hypothesis cannot be categorically rejected is clear from the above chart (text-figure 5) accompanying Russell's article. It shows that a sarcoma endowed with the power of

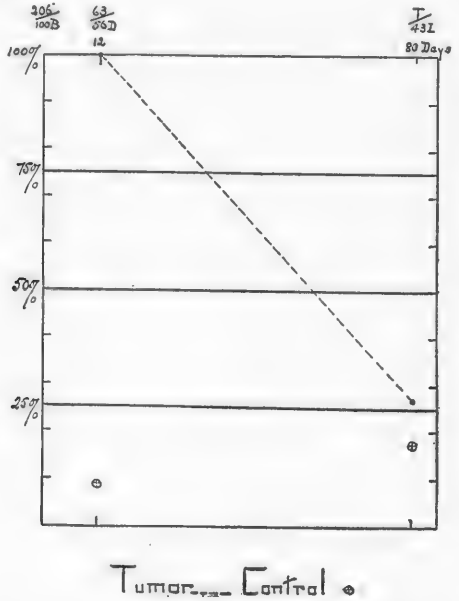
producing the refractory condition is able to do so against a carcinoma capable, under normal circumstances, of progressive growth, if the two be inoculated simultaneously; the author states, furthermore, that a certain carcinoma was discovered to be possessed of similar powers. For the present purpose, both tumors may be considered merely as mouse tissue able rapidly to evolve the refractory condition. As the carcinoma which was suppressed in Russell's



TEXT-FIG. 6. Curve showing the extent and duration of the resistance induced in mice by mouse kidney.

experiment had probably become vascularized by the third day after implantation, and as there is but little or no resistance present at this time according to text-figures 3 and 4, it is not improbable that immunity may be able to defeat a graft which has but recently become established. One accordingly inclines to accept the second explanation as the more satisfactory, reserving its application, however, to the first few days in the history of the graft, and to the

critical period between the third and fifth days when immunity is rising rapidly. This explanation does not nullify the objection brought forward in a previous paper[12] against confounding grafts which perish immediately in animals that have been immune for ten days or more, with those able to proliferate for a time before they are finally vanquished.



TEXT-FIG. 7. Curve showing the rate of disappearance of the resistance induced in mice by a transplantable carcinoma which undergoes spontaneous absorption in a large number of animals. These mice were relieved by operation of carcinoma 206, eleven days after inoculation, and on the following day were tested with carcinoma 63. Eighty days after the inoculation of 206, they were tested with carcinoma T. The controls were normal mice.

Text-figure 6 shows the extent and duration of the resistance resulting from immunization with kidney. The immunity does not occupy the high level attained by embryo skin, but more nearly approximates the curve for spontaneous tumor. It is more than

[12] Woglom, W. H., *Fifth Scientific Report of the Imperial Cancer Research Fund*, 1912, 43.

probable that, instead of rising, the curve would continue to fall after the thirty-eighth day, were exact conditions capable of attainment.

In order to test the duration of the refractory condition conferred by a transplantable tumor, certain mice which had been relieved by operation of carcinoma 206 (a growth producing a high degree of immunity) eleven days after its inoculation and of which 100 per cent. had proven themselves capable of resisting implantation with adenocarcinoma 63 on the day following operation, were inoculated with adenocarcinoma T eighty days from the beginning of the experiment. At this time, as text-figure 7 shows, the immunity had fallen to 27 per cent., at which point, allowing for experimental error, it may be said practically to have vanished. It is highly improbable that the unsuccessful inoculation with tumor 63 either increased or prolonged the resistance originally conferred by carcinoma 206.

As the percentage of resistance in the untreated control mice varied throughout the whole series between 0 and 27 per cent., the charts suggest the futility of drawing conclusions in cases where the difference between treated mice and their normal controls is less than 25 per cent.

CONCLUSIONS.

The foregoing experiments demonstrate that the immunity induced in mice by preliminary treatment with tumor or certain of the normal tissues reaches its maximum at about the tenth day, after which it gradually diminishes, probably to disappear after the lapse of about eighty days. It is significant that the curves with the three tissues, spontaneous tumor, kidney, and embryo skin, should parallel each other so closely, and the occurrence renders extremely probable the view, previously expressed by Russell[13] and by Woglom[14] after an examination of very young grafts in immune mice and rats respectively, that the resistance elicited in each case is similar. It is evident, however, that there is a difference in the degree to which it is developed.

[13] Russell, B. R. G., *Third Scientific Report of the Imperial Cancer Research Fund*, 1908, 341.

[14] Woglom, W. H., *loc. cit.*

TOXIC SUBSTANCES PRODUCED BY PNEUMOCOCCUS.*

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There is much obscurity concerning the relation between the growth of pneumococci in the body and the symptoms and death induced in animals and in man. The view that bacteria act mechanically was long ago held to be inadequate and incorrect. The discovery of toxic substances produced by the growth of certain bacteria outside the body, notably by *Bacillus diphtheriæ* and *Bacillus tetani,* gave support to the supposition that the effects of the growth of all bacteria in the body were due to poisons arising through the metabolic activities of the cells. Since the poisons, however, could not be demonstrated in the culture fluids of other bacteria, this supposition has not received final proof. The demonstration by Pfeiffer that certain bacteria killed by heat or other harmful agents are toxic, gave support to the view that the effects produced in the body by the growth of bacteria are not due to the products of growth of the bacteria, but to toxic substances set free upon their death and dissolution. The attempt to demonstrate these so called endotoxic substances in a large number of bacteria, however, has not been successful, and in the cases where they have been demonstrated, the exact relationship of the poisons to the symptoms and death induced has not yet been rendered perfectly clear.

These theories, however, do not exhaust the conceivable ways in which the growth of bacteria within the body may exert harmful effects, and it is possible that the effects may not be due to substances which may be isolated, or even be detected by injection into the animal body.

Nevertheless, at the present time, the only hope of arriving more closely at a conception of the process seems to be in the iso-

* Received for publication, August 1, 1912.

lation, from the bacteria or their cultures, of substances having a similar effect to that of the living bacteria themselves.

Thinking that possibly such substances might be present in excess in the fluids of the infected animal, a series of experiments, of which the following is typical, were undertaken.

A rabbit was inoculated with an overwhelming dose of virulent pneumococci. Just before death, the animal was bled to as near complete exsanguination as possible, the serum was removed and passed through a Berkefeld filter in order to remove the bacteria, and as quickly as possible this filtered serum was injected into the veins of a normal animal.

The following is a protocol of one of these experiments:

Rabbit 7.—Weight 2,870 gm. Temperature 39° C.

Feb. 9, 1912, 12:05 P.M. Injected intravenously an emulsion in salt solution of the bacteria obtained by centrifugalization from 1,000 c.c. of a 24 hour bouillon culture of pneumococcus I.4[2].

12:15 P.M. Bowels move actively. 1:10 P.M. Temperature 40° C. 2:10 P.M. Temperature 39.8° C. 3.10 P.M. Temperature 39° C. 4:10 P.M. Temperature 38.4° C. 5:00 P.M. Animal moribund. Animal bled as completely as possible. Blood allowed to clot, serum obtained by centrifugalization. Serum passed through Berkefeld filter under pressure.

Rabbit 8.—Weight 1,600 gm. Temperature 38.2° C.

Feb. 9, 6:30 P.M. Injected into ear vein filtered serum obtained from rabbit 7.

10:30 P.M. Temperature 39.6° C. Animal shows no symptoms.

Feb. 10, 10:00 A.M. Temperature 39° C. Weight 1,600 gm. Animal appears well. 6:00 P.M. Temperature 39° C.

Feb. 11, 10:00 A.M. Weight 1,620 gm.

During the succeeding days a record was kept of the weight. It lost slightly, weighing on February 24 1,510 gm. No other changes were noted.

Other similar experiments were conducted on a series of rabbits, but in no case were acute toxic symptoms noted, nor were chronic changes, as determined by loss of weight, observed. It therefore became evident that if the symptoms of infection by pneumococcus are due to soluble toxins formed in the serum of the infected animal, they are fixed by the tissues as rapidly as formed, or are extremely labile and so lose their toxicity on removal from the body, or possibly are held back with certain of the proteins during filtration.

Attention was then directed to the production of toxic substances by the action of immune serum and complement on pneumococci.

Friedberger,[1] in his study of the nature of anaphylaxis, has shown that by

[1] Friedberger, E., *Ztschr. f. Immunitätsforsch., Orig.*, 1909, iii, 692; 1909-10, iv, 636.

the action of complement on the precipitate formed by the addition of immune serum to the serum antigen, a toxic substance is formed which, on injection into a guinea pig, produces acute symptoms and death, apparently identical with the symptoms and death occurring in anaphylaxis due to the second injection of egg albumin or serum. To the toxic substance contained in the albumin-immune-serum-complement mixture, Friedberger gave the name anaphylatoxin. He considered that this experiment gave the final proof of the antibody reaction in anaphylaxis. Later he[2] showed that a similar toxic substance appeared when bacteria were treated with immune serum and complement outside the body. This "Bakterien-anaphylatoxin" he considered identical with the anaphylatoxin produced from serum and identical with the substances formed within the body on the second injection of protein, to which hypothetical substances he considers the symptoms due. Friedberger thinks the symptoms in infection are due to this substance set free in the body. He defines infection as a "mild, protracted form of anaphylaxis" and anaphylaxis as an "extreme, acute form of infection." Therefore it is not necessary to assume any specific bacterial toxin in any infection, but Friedberger thinks in all infections the symptoms are induced by the same poisonous substance, and the different manifestations of the different infections depend only on different modes of infection, localization, etc., of the different microörganisms.

Neufeld and Dold[3] were able to repeat the experiment of Friedberger, using pneumococcus as antigen. We have also repeated this experiment with pneumococcus. Since no essential differences in the results from those of Neufeld and Dold and of Rosenow[4] were found, the protocols of the experiments are not given.

At least three different views as to the origin of the toxic substance in anaphylaxis have been advanced. Vaughan and Wheeler[5] first showed that by the action on protein of alkali in boiling alcohol, substances were formed which, when injected into animals, produced acute toxic symptoms and death. They suggested that anaphylaxis is due to an analogous splitting of the protein, in this case the splitting of the injected protein occurring in the body as a result of the action of ferments or antibodies which have developed as an immunity response to the first injection.

On the other hand, it has been maintained by Nicolle, Weichhardt, Wolff-Eisner and others that the facts may be best explained by assuming that the antibody reaction does not result in a splitting of the protein, but in the case of bacteria, at least, in a lytic action, resulting in the setting free of preformed toxic substances contained within the bodies of the bacteria, endotoxins.

Finally, Wassermann and Keysser[6] hold that the toxic substance does not arise from the antigen, but is a product of the ambocepter of the serum. They

[2] Friedberger, E., and Vallardi, C., *Ztschr. f. Immunitätsforsch., Orig.*, 1910. vii, 94.

[3] Neufeld, F., and Dold, H., *Berl. klin. Wchnschr.*, 1911, xlviii, 55.

[4] Rosenow, E. C., *Jour. Infect. Dis.*, 1911, ix, 190.

[5] Vaughan, V. C., and Wheeler, S. M., *Jour. Infect. Dis.*, 1907, iv, 476.

[6] Keysser, Fr., and Wassermann, M., *Ztschr. f. Hyg. u. Infectionskrankh.*, 1911, lxviii, 535.

think that the so called anaphylatoxin may be produced by the interaction of inactive substances, as *kieselguhr,* with immune serum, in this case the inactive substance taking the place of the bacterial antigen.

It is impossible here to review all the evidence bearing on this subject, but the experiments to be detailed below will be considered as having a bearing on these various theories.

Neufeld and Dold[7] showed that in order to produce similar toxic substances from bacteria, the presence of serum is unnecessary, but that by simple extraction of bacteria in salt solution containing 0.1 per cent. of lecithin, such toxic substances could be obtained. Rosenow[8] then showed that if pneumococci are merely placed in salt solution and allowed to remain in this solution for forty-eight hours at 37° C., the extract so formed is toxic and on injection intravenously into guinea pigs, symptoms and acute death, like that seen in serum anaphylaxis, result. The nature of the change occurring in the bacteria which renders them toxic has been considered by Rosenow to be a true autolysis. He thinks that, owing to the activity of the ferments contained in the bacterial cell, self digestion occurs and the products of this protein digestion are toxic. He thinks an identical or analogous change occurs within the body of the infected host. Upon the property of self digestion, according to him, depends the virulence of the infecting organism. If the organism autolyzes readily, it is therefore virulent; if it autolyzes only slightly, little of the poison is set free and the organism possesses slight virulence.

Since the symptoms produced by the injection of anaphylatoxin or bacterial extracts resemble very remotely those occurring during the pneumococcus infection, it is not certain that this poison has any relationship with the symptoms of the natural infection. Nevertheless, the production of a toxic substance from the bodies of pneumococci is of great importance since, as is well known, the bodies of pneumococci killed by heat are slightly toxic under ordinary circumstances, and large amounts may be injected into susceptible animals, namely, the rabbit and guinea pig, without producing marked effects. This poisonous extract may be of great importance in the pathogenesis of the disease, but in order to establish this, it will first be necessary to learn more concerning the nature of the substance and how it is produced.

Hence experiments were undertaken to confirm or disprove the observations of others as to the main fact, and secondly, by modification of the conditions, to learn how toxic extracts may uniformly be prepared, in order that they may be studied further.

[7] Neufeld, F., and Dold, H., *Berl. klin. Wchnschr.,* 1911, xlviii, 1069.
[8] Rosenow, E. C., *Jour. Infect. Dis., loc. cit.*

EXPERIMENTS TO DETERMINE THE TOXICITY FOR GUINEA PIGS OF EXTRACTS OBTAINED BY EXTRACTION OF PNEUMOCOCCI IN SALT SOLUTION.

The first set of experiments consisted in determining the toxicity of extracts obtained by keeping our stock organism, designated as N–I, in salt solution for various lengths of time. This organism was obtained from Professor Neufeld and was originally isolated from the sputum of a patient with pneumonia. It is the one which has been largely employed by him in his experiments and in the production of his immune serum. It has been frequently passed through mice and possesses high virulence for these animals, 0.000001 of a cubic centimeter of a bouillon culture uniformly killing one of these animals in less than twenty-four hours. It readily retains its virulence on artificial culture medium, and for this reason it is especially suitable for experimental work and has therefore been largely employed in our laboratory. It is a typical pneumococcus, autolyzes readily in salt solution and is easily soluble in bile. The cultures used in this set of experiments were, in most instances, the first or second passages from mice, though in some cases they had been on artificial medium for as many as six transfers.

In all these tests the organisms were grown in bouillon and were removed from the medium by centrifugalization in order to make sure that we were dealing only with the bodies of the bacteria and not with substances derived from the culture medium. If solid medium is employed, it is almost impossible to obtain the bacteria free from small particles of the medium which may not only furnish toxic substances, but, unless the extract be carefully centrifugalized, when injected into the circulation of animals, may cause death by mechanical stoppage of vessels. The objection to the use of solid medium is especially important in the case of blood or serum agar. The presence of small amounts of the complex blood or serum in the mixture renders the analysis of the phenomenon difficult since it cannot be stated with certainty that the effects obtained are due primarily to pneumococci. Wassermann and others have claimed that shock and death like that seen in anaphylaxis may be due to the injection of foreign serum alone.

The culture medium employed in these experiments was a plain bouillon reaction, approximately + 0.6 to phenolphthalein. In this medium pneumococci grow well, causing a dense uniform clouding of the medium in eighteen to twenty-four hours. At this time the medium still reacts faintly alkaline to litmus. The number of bacteria per cubic centimeter in a twenty-four hour culture of N–I is usually between 250,000,000 and 270,000,000. Since this number was determined several times, the number of bacteria injected is usually designated as those contained in a given number of cubic centimeters of the culture medium. From this a fair idea of the actual number of bacteria injected may then be calculated.

The bacterial bodies having been sedimented by centrifugalization, the supernatant fluid is carefully and completely pipetted away. In the first experiments the salt solution was added directly to this sediment and an emulsion was made by means of a pipette. In all the later experiments, the bacterial bodies were washed in 0.85 per cent. salt solution and again thrown down in the centrifuge and the emulsion made from these washed bacteria. It was found by experiment that moderate amounts of the medium alone may produce considerable effects in the injected animal,—even death, if larger amounts are employed. The symptoms are probably caused by peptone. It is extremely important in experiments of this kind that effects are not ascribed to toxic substances derived from the bacteria, when they are really due to peptone or other substances contained in the medium. After adding the desired amount of salt solution to the bacterial sediment, a uniform emulsion was made by means of a pipette and this emulsion, in a loosely stoppered bottle, was placed in a thermostat or on ice for the desired length of time.

In certain of the experiments the emulsion was covered by a layer of ether, as advised by Rosenow, but in the larger number this was not done. That lysis of the bacteria goes on more rapidly or completely when ether is added was not confirmed. Moreover, what is more important, the injected animals may show marked effects due to the ether alone.

Various concentrations of bacteria in the salt solution used for the extract were employed, the same culture being employed in making extracts of various concentrations. In most of the extracts, 1 c.c. represented the bacteria from 10 to 20 c.c. of bouillon culture, but extracts of lesser and greater concentration were also employed. Usually 6 to 8 c.c. of the extract were injected, but as much as 10 c.c. and as little as 1 c.c. were also inoculated. The extracts injected represented the bacterial bodies obtained from as little as 6 c.c. to as much as 180 c.c. of bouillon culture, or 1.5 billion to 45 billion bacteria. Most tests were made with doses of extract representing about 10 to 20 billion bacteria. After standing in the incubator for twenty-four hours, the fluid usually clears and the bacteria fail to stain entirely by the Gram method, and stain faintly or not at all with the ordinary dyes. Frequently even after autolysis is said to be complete, the forms of the bacteria in the centrifugalized sediment may still be made out when the preparation is stained with the Manson stain. Most extracts were allowed to remain at 37° C. for from twenty-four to forty-eight hours. Extracts were tested that had been kept at 37° C. for as short a time as eighteen hours and as long as six days. Extracts kept on ice from

twenty-four hours to as long as twenty-five days were tested also. Again extracts were used that had been kept at 37° C. for from twenty-four to forty-eight hours and then for various lengths of time on ice. No special effort was made to determine the extent of lysis by a determination of soluble nitrogen, or by determining the changes produced in the rotation of polarized light by the solutions. The tests we have made with the polariscopic method have shown that if changes occur, they are so slight as to be negligible. Certain of the changes recorded by others and on which stress has been laid, have been so slight as to be within the limits of error of the best instruments. The changes undergone by the emulsion are by no means entirely proteolytic, and to employ this method, under the given conditions, as an indication of proteolytic cleavage, is a doubtful expedient.

Guinea pigs were used in making the tests in this set of experiments. At first the guinea pigs employed weighed 250 to 340 gm., but in all the later experiments, the animals were smaller, weighing between 200 and 250 gm. The injections were made into the external jugular vein, and usually 4 to 8 c.c. of fluid were injected, but in a few instances as much as 10 c.c. were injected. Of 144 animals 5 showed immediate symptoms following the injection which could be clearly ascribed to the incomplete removal of ether from the extract. In 90 there were no symptoms at all, or symptoms so slight that it did not seem justifiable to consider them as representing more than the shock due to the operation and injection of the considerable amount of fluid. Following the injection certain animals showed some tremor, the hair becoming ruffled and the animal appearing quiet and sick. In 26 instances the symptoms were described as of moderate severity and could possibly be considered analogous to the mild shock seen in anaphylaxis. The animals frequently coughed, had somewhat labored breathing, and marked tremor. In only 23 animals, however, were symptoms seen which could be definitely considered as resembling those seen in true anaphylactic shock. In most of these the symptoms very closely resembled those seen in true anaphylaxis. The animals coughed, voided urine, had marked dyspnea, and in many cases convulsions.

Seven of these animals died acutely within ten minutes. Where autopsy was performed quickly, the heart was found still beating and the lungs were usually voluminous, though in only one instance did the lungs remain distended, taking the shape of the chest and being firm and dry, as seen in typical anaphylactic death. Small focal hemorrhages were seen in the lungs and in two cases subepicardial hemorrhage.

In the seven instances the symptoms and autopsy findings may therefore be said to resemble closely those seen in anaphylaxis, and if occurring in guinea pigs following the second injection of protein, would undoubtedly have been considered as typical anaphylactic shock.

Of the other 16 animals showing marked symptoms, 10 recovered. Of the remaining 6, 1 died in one and a half hours, 1 in three and a half hours, 1 in twenty-four hours, and the others in from eight to twelve hours. In addition

to the 5 animals which died within twelve hours, there were 51 animals which showed no, or only moderately acute, symptoms, but which died within twelve hours. These 56 animals presented fairly characteristic symptoms, and at autopsy showed lesions such as have been described as occurring in late anaphylactic death. Even where no marked acute symptoms arose, they all had some dyspnea and coughing and appeared quite sick within an hour of the injection, the hair being ruffled and marked tremors occurring. Many passed bloody urine, others showed a slight bloody frothy discharge from the nostrils; and after periods varying from one and a half to twelve hours they died, frequently in convulsions. At autopsy the peritoneum contained a very little blood-tinged fluid or large amounts of clotted blood. The bladder was frequently distended with dark bloody urine. Focal hemorrhages existed in the wall of the stomach and intestine. Similar hemorrhages occurred in the intestine, mainly about the cecum and adjacent portions but sometimes throughout. The lungs were moderately distended and usually dry, although sometimes edematous, and focal hemorrhages, from pin-point size up to 2 to 3 mm. in diameter, were scattered throughout almost constantly. Frequently there were well marked epicardial hemorrhages. Cultures and films made from the heart's blood showed in some cases the presence of a moderate number of organisms; in others the cultures were sterile. That the symptoms and death were not due to infection was rendered most probable by the fact that in many cases no organisms could be demonstrated and also because in guinea pigs, no matter how severe the degree of infection, death rarely occurs within twelve hours. Moreover, the pneumococci in this series of experiments were not very virulent for guinea pigs. Undoubtedly in many of the extracts living organisms were present, but the fact that 71 out of the 144 animals recovered, many of them having received large injections of concentrated extracts, shows that the number of living virulent organisms in the extracts was comparatively small. It would seem, then, that these 56 animals died of intoxication induced by substances contained in the extract.

In the report by Dold dealing with the intoxication of guinea pigs by extracts of pneumococci in lecithin containing salt solution, it is noted that most of the animals did not die acutely, but in from one to six hours. Notwithstanding this late death, they are considered to have shown the features of anaphylactic death. Undoubtedly the kind of intoxication and death occurring in the fifty-six animals described in our series corresponds to that described by Dold.

An attempt to bring the symptoms and kind of death into relation with any of the variables in the preparation of the extract or mode of injection has been fruitless. Some of the animals dying acutely were injected with extracts of low concentration, and others with extracts of high concentration. The same is true of the animals dying late. Nor did the period of autolysis seem to have any

effect on the result. One of the animals dying acutely had been injected with extract kept forty-eight hours at 37° C., and four days on ice; another with extract kept forty-eight hours at 37° C., and five days on ice; another with extract kept forty-two hours at 37° C., and four days on ice; two others with extracts kept forty-eight hours at 37° C., and the remaining two with extracts kept for twenty-four hours at 37° C. Frequently, it was impossible to reproduce the phenomena, even though the conditions were exactly identical so far as could be determined. In two of the animals dying acutely, a few drops of blood were allowed to flow into the syringe and mix with the extract in the syringe before injection. It was thought that some reaction might have taken place between the small amount of blood and the extract. This procedure, however, was repeated in eight other guinea pigs without producing acute death in any case. In one instance, one cubic centimeter of fresh blood was added to the extract before injection. Seven guinea pigs were injected with extracts to which had been added various amounts of fresh guinea pig complement, and the mixture had been kept at 37° C. for from one to two hours. Acute death occurred in only one of these animals. This was quite like that occurring in anaphylactic shock and occurred in an animal which had been injected with three cubic centimeters of extract plus four cubic centimeters of complement, and the mixture kept at 37° C. for two hours.

The result of this set of experiments was therefore inconclusive, in so far as finding a means of producing an extract that would uniformly kill guinea pigs with symptoms like those seen in anaphylaxis. Although seven animals died acutely presenting symptoms resembling acute anaphylactic death, and fifty-six died within twelve hours presenting features resembling those seen in the more chronic forms of anaphylactic death, the results were not constant and striking enough to permit a determination of the exact conditions under which extracts producing acute death may be constantly obtained.

It was thought that possibly the failure to obtain more constant and striking results might be due to the race of organism studied, and consequently a series of tests was made with extracts prepared

from two other races. Both the organisms were obtained from pneumonic patients and had high virulence for mice. The extracts were tested on forty guinea pigs, none of which died acutely. Eight showed moderate or severe symptoms following the injection, and eighteen died within twelve hours. At autopsy these showed conditions resembling those previously described as present in the animals dying late.

Extracts were also prepared from cultures of pneumococci made in bouillon directly from the blood of pneumonia patients. Two extracts made from two races of pneumococci (Davis O′ and Baron O′) were tested, but the results did not differ from those previously mentioned.

Extracts were also prepared from two cultures kindly sent us by Dr. Rosenow. The following is a brief report of the results obtained with them.

Culture 622^{4} (*R–I*).—Blood agar. Received Dec. 12, 1911. This organism was "isolated from the blood on the second day of the disease in a case of lobar pneumonia, and has been passed through four guinea pigs."

Three extracts, prepared as in previous experiments, were tested on four guinea pigs. None showed marked symptoms. One died within twelve hours and showed the lesions previously described.

Culture 602^{31} (*R–II*).—Blood agar. Received Dec. 12, 1911. This organism was "originally isolated from consolidated lung after death and has been passed through thirty-one guinea pigs."

Two extracts were prepared in the usual way and tested on four guinea pigs. None showed marked symptoms. All recovered.

A test of the virulence of this organism (602^{31}) showed that while it had high virulence for mice, its virulence for guinea pigs, in spite of the thirty-one passages, was not very high. An attempt was therefore made to raise its virulence for guinea pigs before retesting the extract. The pneumococci were passed through a series of guinea pigs, inoculating directly from the abdominal cavity of one guinea pig to that of the next. After passage through twelve guinea pigs in this way, the virulence had been somewhat increased.

Cultures were made at various times from the peritoneal cavity of the dead guinea pigs and extracts were made from the cultures. The extracts were made from cultures of the first, sixth, seventh, twelfth, and thirteenth passages, in plain broth, and in 2, 6, and 8 per cent. glucose broth. All were covered with ether during autolysis. The extracts were tested on sixteen guinea pigs, none of

which died acutely, and which showed symptoms like those seen in anaphylaxis. Eight guinea pigs showed practically no early symptoms whatever. Three showed quite marked immediate symptoms of collapse, which seemed to be associated with incomplete removal of the ether. Only two showed marked symptoms which were like those seen in acute anaphylaxis. Six recovered. Eight died within twelve hours showing symptoms and autopsy findings like those seen in the similar group with other extracts. One died in twenty-four hours.

Hence, so far as tested, these extracts did not differ materially in their effect from those prepared from the cultures which were less virulent for guinea pigs.

The virulence for guinea pigs of the N–I culture used in the previous experiments was low. While 0.000001 of a cubic centimeter of the twenty-four hour culture was uniformly fatal for mice within twenty-four hours, as large quantities as the bacteria from five cubic centimeters of culture did not kill guinea pigs uniformly. Of six guinea pigs injected with 1.1 cubic centimeters, four recovered and two only died after eight days.

An effort was made to increase the virulence of this race for guinea pigs in order to determine whether the inconclusive results might be due to the low virulence for these animals. The same method was employed to raise the virulence of this organism as that used in the case of organism R–II. After passing through the abdominal cavities of fourteen guinea pigs, a culture was made in 6 per cent. glucose bouillon and an extract prepared. This was tested in five guinea pigs. One showed marked symptoms and died acutely, in a manner resembling acute anaphylactic death. The others recovered.

RESULTS OF THE STUDY OF SALT SOLUTION EXTRACTS.

As compared with the reports of others, the results of the study of the toxicity of extracts of pneumococci in salt solution have been disappointing. The observations of Dold would not lead one to expect to find the extracts acutely toxic with any great regularity, while those of Rosenow would indicate that acutely toxic extracts are obtained without difficulty. Of the 213 guinea pigs we injected with salt solution extracts of the several races of pneumococci, only eight died acutely. In these the symptoms present and the autopsy

findings resembled those present in anaphylaxis. Eighty-three of the animals died in from one to twelve hours under fairly characteristic conditions, such as are sometimes seen in sensitized animals dying several hours following the second injection of a protein. So far we have not found the exact conditions upon which the development of toxicity in the salt solution extracts depends. It is, of course, possible that the varieties of guinea pigs are responsible for the irregular results. Rosenow[9] states in his last communication that the susceptibility of guinea pigs to the action of extracts is greatly increased by starvation. We have made no changes in the regular feeding of the animals tested. Further study may show that factors which we have overlooked in the preparation of the medium or mode of injection, are responsible for the failures, but without a knowledge of these factors any quantitative study of the problem will be impossible.

STUDY OF PERITONEAL WASHINGS.

Since the salt extracts of pneumococci did not show as high toxicity as was anticipated, it was held possible that in the peritoneal cavity the solution of the bacteria might go on at a more rapid rate, from which cavity solutions might be obtained of greater and more constant toxicity.

Although the experiments on rabbits, mentioned early in the paper, to determine the toxicity of the blood of infected rabbits proved negative, yet it seemed possible that in the case of peritoneal infection, the toxin might not be so quickly fixed, or might be formed in such overwhelming amount that it might be found later. Friedberger and Nathan[10] have reported finding "anaphylatoxin" in the peritoneal cavities of guinea pigs into which *Bacillus prodigiosus* had been injected. Rosenow also states that the peritoneal exudates following the injection of pneumococci are toxic.

The guinea pigs from which the exudate was obtained were infected by intraperitoneal injection. As soon as possible after death the peritoneal cavity was opened and washed out with a small amount of normal salt solution.

[9] Rosenow, E. C., *Jour. Infect. Dis.*, 1912, xi, 94.

[10] Friedberger, E., and Nathan, E., *Ztschr. f. Immunitätsforsch., Orig.*, 1911, ix. 444.

Usually 10 to 15 c.c. sufficed to obtain sufficient fluid for the subsequent injection. After mixing thoroughly with the small amount of exudate usually present, the fluid was removed by a pipette and placed in a powerful centrifuge in which the cells and the larger number of bacteria were removed. The clear fluid was injected into the jugular vein of the animals used in the test. Since it was possible that the pneumococcus which had grown in the animal might be more susceptible to lysis or yield toxin more readily than those grown on artificial media, the sediment obtained on centrifugalization of the peritoneal washings, consisting of bacteria and a varying number of cells, had added to it 10 to 15 c.c. of salt solution and the resulting emulsion was placed at 37° C. for twenty-four to forty-eight hours, after which it was again centrifuged and the supernatant fluid injected into the vein of guinea pigs. Peritoneal washings were obtained from nine guinea pigs which had been infected with pneumococcus R–II. In two instances sufficient washings were obtained for testing each on two guinea pigs, so that in all eleven guinea pigs were injected.

Of these eleven animals, eight showed immediate symptoms like those seen in anaphylaxis, two showed but slight symptoms following the injection. One animal died during the injection. In this case the fluid was thick and viscid and death may have been due to mechanical causes. Of the remaining ten animals, four died within a few minutes with typical features of anaphylactic death and with characteristic autopsy findings. The other six all died in less than twelve hours, and at autopsy, findings, previously described in other animals dying subacutely, were found. That infection was not primarily the cause of death in these animals is shown by the fact that cultures and smears from the heart's blood were usually negative. Of course most of them would undoubtedly have died later from infection, but it seems probable that in these instances death was due to toxic substances transferred from the previously infected animals.

Seven animals were injected with the extract obtained by allowing the sediment to undergo autolysis in salt solution, as previously noted. The results were not nearly so striking as those in which the peritoneal washings were immediately employed.

The direct injection of peritoneal washings into the veins of other guinea pigs gave results which were important and striking. That four out of the ten animals had acute shock and death simulating that seen in anaphylaxis indicates that the development of the toxic substance must be much more constant in the abdominal cavity of the guinea pig than in the test-tube, if we assume that the same

process is active in the two cases. However, in the animal body the conditions are much more complex. In order to obtain an active extract under as simple conditions as possible, further efforts were directed to prepare it outside the body.

STUDY OF CHLOROFORM EXTRACTS.

The toxic effect of pneumococcus is not necessarily due to digestion products of the bacteria, as has been considered to be the case, but may be caused by preformed substances set free on the disintegration of the bodies of the bacteria, the essential process being, therefore, a form of plasmolysis, rather than of autolysis. Various methods were employed in an endeavor to throw the bacterial bodies into solution rapidly under conditions in which autolysis could, with reasonable certainty, be excluded.

Of the procedures, the only one giving any degree of success consisted in the use of chloroform, by which method we were able to obtain fairly constant solutions of the bacterial bodies. The method, however, is troublesome. The mixtures of chloroform and emulsion do not always become clear, and the injection of the solution into the veins of animals did not give constant results.

STUDY OF BILE EXTRACTS.

We next studied the solution of pneumococci obtained by means of bile. It was first shown by Neufeld[11] that pneumococci, when treated by bile or solutions of bile salts, readily undergo solution, and the emulsion, which was previously cloudy and opaque, becomes clear and translucent and on staining, the form of the bacterial bodies can no longer be made out. Although Rosenow states that ". . . complete lysis of pneumococci in weak solutions of bile salts (0.5 per cent.) . . . interferes with the production of anaphylatoxic substance . . .," preliminary experiments performed by us showed that solutions of pneumococci in bile salts, when injected intravenously, were toxic for guinea pigs. In making the solutions, a 2 per cent. solution of sodium cholate in normal salt solution was employed. It was found, however, that with this low dilution of the

[11] Neufeld, F., *Ztschr. f. Hyg. u. Infectionskrankh.*, 1900, xxxiv, 454.

bile salt, clearing of various solutions did not always occur with equal rapidity, some solutions clearing more rapidly and others not at all.

An experiment was therefore undertaken to determine the effect of dilutions of sodium cholate on the lysis, the number of bacteria and the amount of sodium cholate being kept constant.

Two sets of tubes were prepared, one set kept at 37° C., the other on ice. It was found that the action of the cholate depends on the concentration of the sodium cholate in the emulsion, rather than on the relation of the amount of the salt to the number of bacteria present. 0.1 c.c. of a 2 per cent. sodium cholate solution was found to be much more efficient in producing solution of the given number of bacteria, when they were in a 1 c.c. emulsion, than even 0.2 c.c. when the bacteria were made into a 10 c.c. emulsion. It was found also that while lysis occurs somewhat better at 37° C., yet complete solution, when the proper mixtures are employed, may be obtained when the entire process is carried out on ice.

Even bearing in mind the above fact, however, solution of pneumococci could not always be readily obtained, even when the proper mixtures were employed. It was found that with bacteria grown in glucose bouillon, even when washed in salt solution, solution did not readily occur. It was found that when pneumococci were grown in plain bouillon and centrifugalized, then washed with the supernatant fluid from the glucose bouillon culture, the organisms, which otherwise readily dissolved, were now insoluble. It was therefore evident that the insolubility was due to something in the glucose bouillon fluid which adhered to or was absorbed by the bacteria. It seemed probable that this was acid, and this was found to be the case. Very small amounts of acid in the emulsion prevent solution of the bacteria, probably owing to the fact that cholic acid, which is insoluble, is set free.

A test was therefore made to determine the optimum reaction for lysis with bile. As is well known, by mixing primary and secondary sodium phosphate, solutions may be obtained which retain to a considerable degree their original reaction, in spite of changes occurring in the medium which would otherwise influence the reaction. By adding mixtures of primary and secondary phosphates in various proportions to the tubes containing bacteria and bile, it was found that lysis goes on most rapidly when mixtures of one

part primary to seven to eight parts secondary phosphate are employed. This gives about the reaction of blood serum.

EXPERIMENTS WITH GUINEA PIGS.

Sixty-three guinea pigs, weighing 200 to 250 grams each were injected with various amounts of the cholate extracts of pneumococci. Forty-nine showed marked symptoms similar to those seen in acute anaphylaxis, one showed moderately severe symptoms, and thirteen showed only slight symptoms or none at all. Of the sixty-three animals, thirty-seven died acutely under conditions resembling those seen in acute anaphylaxis, fifteen died in from one half to twelve hours and showed hemorrhages and other features previously described as occurring in animals dying after a few hours, one animal died during the injection,—the cause of death could not be determined,—while ten recovered. It will thus be seen that a large proportion of the animals died acutely in the manner seen in acute anaphylaxis, far more deaths occurring than with extracts prepared by extraction in salt solution. It must be remembered that these results were obtained by employing extracts prepared in various ways. The results would be much more striking if they included only animals injected with extracts prepared in the manner which we now know is most efficient for producing a toxic effect.

In the above series, the extracts injected contained only small amounts of sodium cholate. One animal dying acutely received but 0.04 of a cubic centimeter of the cholate solution. All but seven of the animals received less than 0.2 of a cubic centimeter of the 2 per cent. solution. Six received between 0.2 and 0.3 of a cubic centimeter, but of these only three died acutely. One received more than this, but it died only after four and a half hours. That the symptoms and death are not dependent upon the sodium cholate was shown, moreover, by control tests in which animals were injected with different amounts of the sodium cholate solution. One received 0.2, one 0.4, and one 0.6 of a cubic centimeter of the cholate solution in eight cubic centimeters of sodium chlorid solution. None of them showed symptoms and none died. It is quite probable that very much larger amounts could be injected without producing symptoms. It was thought possible that the reaction

might be due to some non-specific combination between the protein and the sodium cholate, of the nature of a physical reaction. But a guinea pig injected with a solution of egg albumin treated with sodium cholate solution showed no symptoms.

The extracts injected contained the substance of varying amounts of bacteria. The race of pneumococcus described as N–I was exclusively employed. After centrifugalizing from the bouillon culture, the pneumococci were washed in salt solution. Some of the extracts were prepared from quite large numbers of bacteria,—as many as those obtained from centrifugalization of 160 c.c. of the culture. The larger number, however, were prepared from the bacteria obtained by centrifugalization of 60 to 80 c.c. of the culture. Positive results were obtained also from smaller amounts. In one instance, acute symptoms and death developed after the injection of an extract prepared from the bacteria obtained from only 25 c.c. of the culture.

The solution of the bacteria was obtained by leaving the mixture on ice or at 37° C. for various lengths of time, usually from one half to several hours. In three instances acute symptoms and death were seen after the injection of extracts that had been prepared in the cold and kept on ice for only thirty minutes before injection. Three positive results were obtained with extracts that had been kept at 37° C. only ten minutes, and in five cases the mixture had been kept only fifteen minutes at 37° C. It is thus seen that the reaction which occurs between the bile salt and the bacteria occurs very rapidly, only sufficient time being required to bring the bacterial bodies into solution. Since by experiment we have learned how to produce this result most rapidly, it has been found that positive results may be obtained almost invariably, by the injection of 6 c.c. of an extract prepared from the bacteria in 50 to 60 c.c. of culture, the mixture having been kept half an hour at 37° C. The best method we have found is the following: 1,000 c.c. of a twenty-four hour culture are centrifugalized, the bacteria are washed once in salt solution, and after the second centrifugalization, the supernatant fluid is removed as completely as possible. Five c.c. of salt solution are now added, and 5 c.c. of a mixture of primary and secondary sodium phosphate solutions in the proportion of 1 to 8; a thick emulsion is thus produced, after which 2 c.c. of the 2 per cent. sodium cholate solution are added and mixed, and the mixture is placed in the water bath at 37° C. for half an hour. Sufficient salt solution is then added to bring the total up to 100 c.c., and after thorough mixing, the extract is ready for injection. The extract need not be centrifugalized unless it contains foreign particles. Six c.c. of such an extract contains the bacterial substance from the bacteria contained in 60 c.c. of the bouillon culture and 0.12 c.c. of the sodium cholate solution.

The symptoms produced and the results obtained are proportionate to the amount of extract injected, though there appear to be some individual differences in sensitiveness in the different guinea pigs. Yet two extracts prepared exactly alike do not necessarily have identical toxicity, a fact depending in part upon the varying

number of bacteria contained in different cultures. Each extract, therefore, has to be independently standardized.

TESTS OF THE BILE EXTRACTS IN RABBITS.

The toxicity of the bile extracts of pneumococci has been further studied by injection into rabbits. Rabbits weighing 700 to 1,200 grams were employed, and the injections were made into the ear veins. A considerable number of the rabbits injected with fifteen to twenty cubic centimeters of the extract died acutely in from two or three to thirty minutes. Of twenty-nine rabbits injected, eleven died acutely, eleven died in from one to twelve hours, and seven recovered or died of infection in from one to three days.

A rabbit dying within a few minutes showed, usually, the following symptoms: After the injection it would be quiet for a few minutes, then would suddenly start to run violently across the floor during which process it usually fell, and frequently would throw itself about on the floor. While on its side it would continue to make running motions with its legs, its head would be drawn back, its eyes prominent, the pupils usually dilated, the animal would cry out, make a few convulsive movements, and then died. When the symptoms were a little less acute, the progression of the disturbance could be better followed. The following protocol describes fairly well what occurred in a typical experiment:

Rabbit 5 E.—Weight 700 gm.

April 5. Injected into the ear vein 15 c.c. of an extract made by treating the bacteria from 200 c.c. of a bouillon culture with 0.7 c.c. of a 2 per cent. solution of sodium cholate. The mixture was kept on ice for thirty minutes before injection. Injection was made at 10:56 A.M. At 10:58 the animal appeared drowsy and showed some muscular weakness. 10:59. Respirations are slower; animal appears sick. 11:01. Marked weakness in legs; animal no longer able to hop about. 11:10. Animal lying on side, not attempting to get up. 11:16. Respirations are shallow, 18 to the quarter, slower than previously. 11:19. Makes jumping motions while lying on side. Heart no longer felt. Respirations shallow. 11:21. Active peristalsis. 11:22. Several gasping respirations. Heart cannot be felt. Pupils dilated. Twitching of muscles noted. 11:23. Animal dead.

Autopsy.—Mesenteric veins markedly dilated. No hemorrhages in wall of intestine or stomach. Stomach firmly contracted. Blood removed from mesenteric vein begins to clot in half an hour; not completely clotted in forty-five minutes. Lungs not especially voluminous; show small areas of emphysema.

Heart: On opening the chest, the left ventricle is seen to be making slow ineffectual beats, the auricles are beating less frequently than the ventricles, then in dissociation. There are no pulsations in the right ventricle unless it be at the tip. The right ventricle is much distended, the muscle opaque. On opening the right ventricle, the wall contracts and becomes crinkled. On scraping, the muscle seems distinctly tougher than usual.

Other animals showed features differing slightly in details from those described. Some of them showed paralysis, beginning in the hind legs, the animals dragging themselves about. In general, muscular weakness was an early condition and death seemed to be due to cardiac, rather than to respiratory involvement. The animals showed in general the features described by Auer as occurring in acute anaphylactic shock in rabbits. A typical animal, seen by Auer, was said by him to behave and to show autopsy findings like those seen in his rabbits.

The animals dying after several hours showed at autopsy changes resembling more those seen in guinea pigs dying late. Focal hemorrhages were an almost constant finding. These were most marked, as in the guinea pigs, in the cecum and adjacent portions of the intestine, in the stomach, and in the lungs. Frequently there was a bloody exudate into the peritoneum and the bladder was filled with bloody urine.

In this series of rabbits, as in the guinea pigs, extracts of various concentration were employed and were prepared by leaving the mixtures at 37° C. or on ice for short periods of time. Typical acute death was obtained with extracts that had been kept at 37° C. for only ten minutes and also from extracts that had been prepared in the cold and kept on ice no longer than thirty minutes. It was finally found that by the injection of fifteen to twenty cubic centimeters of the extract prepared as described on page 660, acute death could almost always be produced. The tolerance of rabbits for sodium cholate was as great as four cubic centimeters of the solution in sixteen cubic centimeters of salt solution without any symptoms whatever. This is a very much larger amount than was injected in any of the experiments, and shows that the effects cannot be due to that substance.

That the result described bears a quantitative relationship to the amount of bacteria injected is shown by the following experiment:

Five rabbits were injected with various quantities of an extract prepared as previously described. One hundred c.c. of extract were prepared from the bacteria in 1 liter of culture medium. One and a half c.c. of sodium cholate solution were used in causing solution which occurred in fifteen minutes at 37° C.

Rabbit No.	Amount of extract injected.	Symptoms.	Result.
1	22 c.c.	Marked	Died, 3 min.
2	20 c.c.	Slight	Died, 4 hrs.
3	15 c.c.	Slight	Died, 11 hrs.
4	10 c.c.	Slight	Died, 12 hrs.
5	5 c.c.	None	Recovered.

Further studies dealing with the properties of the bile extract will be reported later. It may be stated, however, that tests of the effect of heating the extract gave the following results:

Guinea pig No.	Extract heated.	Result of injection. Symptoms.	Final results.
1	60–65° C. for 1 hr.	None	Recovery.
2	55° C. for ½ hr.	None	Recovery.
3	55° C. for ½ hr.	Very slight	Recovery.
4	55° C. for ¼ hr.	Very slight	Recovery.
5	45° C. for 1 hr.	Marked	Died, 30 min.

Controls with the extracts employed unheated showed marked symptoms and acute death.

Rosenow states that the toxic substance in salt solution extracts is destroyed by heating for one half hour at 60° C., but Dold[12] that the toxic extracts are quite resistant to heat; they are weakened by two hours heating to 56° to 58° C., but are not destroyed. The results of our tests of a number of bile extracts seem to show conclusively that they are thermolabile. Possibly the reason that the bodies of bacteria killed by heat are not toxic is because the thermolabile toxic substance is destroyed.

The study of the extract of pneumococcus in bile solution has shown that its toxicity for rabbits and guinea pigs is greater and more constant than is the extract in salt solution. Further work will be required to show its relation to the poison responsible for the symptoms of pneumococcus infection in animals and of lobar pneumonia in man. The fact that poisons having a similar action are found in the peritoneal exudate of infected animals suggests that these poisons may play a part in pneumococcus infection.

[12] Dold, H., Das Bakterien-Anaphylatoxin, Jena, 1912.

These observations also have a bearing on the present theories of anaphylaxis. The fact that the effects produced by this poison, both in guinea pigs and rabbits, are similar to those produced by the second injection of protein, suggests that the poisons concerned are of a similar nature, though this is not necessarily the case. In the present instance there is evidence that the poison does not arise as a result of protein digestion, since it may be set free within half an hour at a temperature of about 4° C. Proteolytic cleavage, due to ferments with which we are familiar, does not occur under these conditions. It is more likely that the toxic substances in the bile extract exist preformed in the bodies of the bacteria and are set free on their dissolution.

CONCLUSIONS.

1. The filtered blood serum of rabbits infected with pneumococci is not toxic.

2. Extracts of pneumococci prepared by keeping emulsions of the bacteria in salt solution at 37° C. for varying periods of time may be toxic, and when injected intravenously into guinea pigs, may produce a train of symptoms followed by acute death resembling that seen in acute anaphylaxis. Such extracts, however, are not uniformly toxic and it has been impossible to discover the exact conditions under which such extracts become toxic.

3. When the centrifugalized peritoneal washings of guinea pigs infected with pneumococci are injected into the circulation of normal guinea pigs, these animals very frequently exhibit symptoms like those seen in acute anaphylaxis, and a considerable proportion of the animals die acutely.

4. When pneumococci are dissolved in dilute solutions of bile salts and the solution resulting is injected intravenously into rabbits and guinea pigs, these animals show with great constancy the same symptoms that are seen in acute anaphylaxis. The solution of pneumococci in bile may occur in ten minutes at 37° C. or in half an hour on ice. This is considered evidence that the toxicity of the solution does not result from digestion of the bacterial protein, but is due to substances preformed in the bacterial cells and set free on their solution. The toxicity of the solution is diminished or destroyed by heating to 55° C. or over.

THE PRESENCE OF PROTECTIVE SUBSTANCES IN HUMAN SERUM DURING LOBAR PNEUMONIA.*

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As long ago as 1891, G. and F. Klemperer[1] showed that the blood of patients who had recovered from an attack of pneumonia might protect rabbits against infection with pneumococcus. Since then Neufeld and Haendel[2] have also demonstrated that such sera may have protective power, not only for rabbits but for mice as well. Other observers have found that this property of post-critical blood serum is not constant, and Strouse,[3] and Seligmann and Klopstock,[4] have even failed to demonstrate it in any instance. The observations previously mentioned, however, seem to indicate that such protective substances may be present at the time of the crisis or afterward.

Considerable speculation has been indulged in, and no little work carried on in order to bring the development of this protective power into direct causal relationship with the crisis in this disease. The crisis is such a clear-cut, striking phenomenon that it would seem that it may depend on some single cause or relatively simple biological reaction, such as the destruction of the bacteria by immune substances or neutralization of toxin by antitoxin. If the blood of animals recovering from the disease constantly contains protective substances, it would be reasonable to assume that the crisis and recovery occur because of the development of such substances.

Observations have also been made to determine the variety of the

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[1] G. and F. Klemperer, *Berl. klin. Wchnschr.*, 1891, xxviii, 833, 869.

[2] Neufeld, F., and Haendel, *Arb. a. d. k. Gsndhtsamte*, 1910, xxxiv, 166.

[3] Strouse, S., *Jour. Exper. Med.*, 1911, xiv, 109.

[4] Seligmann, E., and Klopstock, F., *Ztschr. f. Immunitätsforsch., Orig.*, 1909–10, iv, 103.

immune bodies developing in the blood, to which the protective power is due. G. and F. Klemperer considered the protective power dependent upon an antitoxic action of the serum. Römer[5] holds the opinion that the chief action is bactericidal; while recently much stress has been laid on the development of opsonins or bacteriotropins in the serum. In the absence of the demonstration of a pneumococcus toxin, the view that the protective power may be due to an antitoxin is purely hypothetical. Even Römer has not been able to demonstrate the bactericidal power *in vitro* of post-pneumonia serum. Virulent pneumococci are so resistant to the action of opsonins, as detected by the ordinary technique, that even artificial immune sera, having far greater protective power than any post-critical sera so far studied, show very slight if any increase in opsonic power for such virulent organisms. By the use of a different technique, Neufeld and Haendel have demonstrated that such post-critical sera may show an increase of their property of favoring phagocytosis, both *in vivo* and *in vitro*. This property, they consider, depends on the presence of bacteriotropins.

In the effort to find an explanation of the crisis, however, it is of great importance to determine definitely whether protective substances appear constantly in the post-critical serum, and whether the time of their appearance bears any relationship to the time of crisis or recovery. The mere demonstration of the occasional presence of protective substances in post-critical sera is not sufficient to permit drawing any conclusions as to their relationship to the phenomenon.

The blood obtained from a series of patients has therefore been studied to determine whether such substances are present, and if so, to determine the time of their appearance. No effort has been made to determine the kind of immune bodies present.

EXPERIMENTAL PART.

Methods.—The sera for the experiments presented in this paper were obtained by venous puncture of patients with typical lobar pneumonia. The blood was allowed to clot, the serum separated,

[5] Römer, P. H., Experimentelle und klinische Grundlagen für die Serumtherapie der Pneumokokkeninfektion der menschlichen Cornea (*Ulcus Serpens*), Wiesbaden, 1909.

and kept in the dark on ice until used. The organism employed in each experiment was obtained, when possible, either from the blood or sputum of the patient whose serum was to be tested. The organism in each instance was isolated before the occurrence of the crisis. It has been found of some service, in procuring pure cultures from sputum, to inject at the same time a small quantity of human serum which seems to inhibit the growth of contaminating bacteria. In case the pneumococcus, when isolated, was of low pathogenicity, the virulence was raised by successive animal passages until a dose of 0.000001 of a cubic centimeter of a broth culture was sufficient to kill. White mice of as nearly the same age and size as possible were used as experimental animals. Twenty-four hour broth cultures fresh from animals were used for infection, and the serum and culture to be tested were mixed in varying quantities in the barrel of a syringe and immediately injected intraperitoneally. The animals were watched for from five to ten days, according to the rate at which the culture killed, and those surviving this time were considered protected. A small number of sera were tested for protective power against a typical laboratory strain of pneumococcus; in all other instances, the sera were tested against homologous strains isolated from the patients.

PROTECTIVE POWER OF HUMAN SERA AGAINST A TYPICAL PNEUMOCOCCUS.

Experiment 1.—Serum 123 was obtained from a patient with typical pneumonia of the right lower lobe. The temperature fell by lysis on the seventh day of the disease. The temperature again rose the next day and remained slightly elevated for about six days. The blood culture was negative. The organism used for infection was pneumococcus Neufeld I, a strain of constant high virulence for animals.

Quantity of culture in c.c.	Quantity of serum in c.c.	Virulence; no serum.	Control serum, chronic endocarditis.	Serum 123, Mar. 23, 1911, 12 hours before crisis	Serum 123, Mar. 24, 1911, 4 hours after temperature reached normal.	Serum 123, Mar. 27, 1911, 3 days after temperature reached normal. Patient again febrile.	Serum 123, Mar. 30, 1911, 6 days after temperature first reached normal. Patient still febrile.	Serum 123, Apr. 3, 1911, 10 days after temperature first reached normal. Patient again afebrile.	Serum 123, Apr. 13, 1911, 20 days after temperature first reached normal. Patient afebrile.
0.1	0.2	D.[6] 21 hrs.	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.	—	D. 21 hrs.	D. 21 hrs.
0.01	0.2	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.	D. 26 hrs.	D. 21 hrs.	—	D. 21 hrs.	D. 21 hrs.
0.001	0.2	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.	—	D. 21 hrs.	D. 26 hrs.
0.0001	0.2	D. 36 hrs.	D. 36 hrs.	D. 36 hrs.	Survived.	D. 21 hrs.	D. 21 hrs.	D. 24 hrs.	Survived.
0.00001	0.2	D. 36 hrs.	D. 26 hrs.	Survived.	D. 56 hrs.	D. 36 hrs.	D. 36 hrs.	D. 26 hrs.	D. 36 hrs.
0.000001	0.2	D. 36 hrs.	D. 36 hrs.	Survived.	D. 36 hrs.	D. 36 hrs.	D. 26 hrs.	D. 23 hrs.	Survived.

[6] D. = died.

Experiment 2.—The serum (405) for this test was obtained from a patient with partial pneumonic consolidation of the left lower lobe. The temperature fell by lysis from the eighth to the ninth day of the disease and remained normal. The blood culture was negative. The protective power of the serum was tested against pneumococcus Neufeld I.

Quantity of culture in c.c.	Quantity of serum in c.c.	Virulence; no serum.	Control serum, syphilis.	Serum 405, Dec. 14, 1911, immediately before beginning of lysis.	Serum 405, Dec. 15, 1911, 6 hours after temperature reached normal.	Serum 405, Dec. 21, 1911, 6 days after temperature reached normal.	Serum 405, Jan. 2, 1912, 18 days after temperature reached normal.
0.1	0.2	D. 18 hrs.	D. 18 hrs.	D. 18 hrs.	D. 18 hrs.	—	—
0.01	0.2	D. 18 hrs.	D. 18 hrs.	D. 18 hrs.	D. 18 hrs.	D. 24 hrs.	D. 18 hrs.
0.001	0.2	D. 18 hrs.	D. 18 hrs.	D. 24 hrs.	D. 18 hrs.	D. 24 hrs.	D. 18 hrs.
0.0001	0.2	D. 18 hrs.	D. 24 hrs.	D. 24 hrs.	D. 36 hrs.	D. 24 hrs.	D. 28 hrs.
0.00001	0.2	D. 36 hrs.	D. 48 hrs.	D. 28 hrs.	D. 36 hrs.	D. 28 hrs.	D. 28 hrs.
0.000001	0.2	D. 28 hrs.	D. 28 hrs.	D. 36 hrs.	D. 48 hrs.	D. 48 hrs.	D. 48 hrs.

Experiment 3.—The patient whose serum (418) was used in this experiment had a short pneumonia, terminating by lysis from the fourth to the sixth day of the disease. The blood culture was negative. Protection for animals was tested against the stock laboratory pneumococcus Neufeld I.

Quantity of culture in c.c.	Quantity of serum in c.c.	Virulence; no serum.	Control serum, syphilis.	Serum 418, Dec. 24, 1911, 2 days before beginning of lysis.	Serum 418, Dec. 29, 1911, 1st day of normal temperature.	Serum 418, Jan. 8, 1912, 10 days after first normal temperature.
0.1	0.2	—	—	—	—	D. 18 hrs.
0.01	0.2	—	—	D. 18 hrs.	—	D. 28 hrs.
0.001	0.2	—	—	D. 18 hrs.	—	D. 18 hrs.
0.0001	0.2	D. 18 hrs.	D. 24 hrs.	D. 28 hrs.	D. 28 hrs.	D. 28 hrs.
0.00001	0.2	D. 36 hrs.	D. 48 hrs.	D. 36 hrs.	D. 36 hrs.	D. 28 hrs.
0.000001	0.2	D. 28 hrs.	D. 28 hrs.	D. 28 hrs.	D. 36 hrs.	D. 36 hrs.

PROTECTIVE POWER OF HUMAN SERA AGAINST HOMOLOGOUS PNEUMOCOCCI.

Experiment 4.—The serum (119) for this experiment was obtained from a patient with typical lobar pneumonia, terminating by lysis on the tenth day, although the temperature remained slightly elevated for six days thereafter. The pneumococcus used for infection was obtained from the patient's blood on the seventh day of the disease. When first isolated, 0.5 c.c. of a twenty-four hour broth culture failed to kill a mouse. The virulence was raised by successive animal passages until 0.000001 c.c. was fatal for mice in forty-eight hours.

Quantity of culture in c.c.	Quantity of serum in c.c.	Virulence: no serum.	Control serum, syphilis.	Serum 119, Mar. 20, 1911, 3 days before beginning of temperature fall.	Serum 119, Mar. 21, 1911, 2 days before beginning of temperature fall.	Serum 119, Mar. 22, 1911, 1 day before beginning of temperature fall.	Serum 119, Mar. 23, 1911, 3 hours after critical drop in temperature.	Serum 119, Mar. 25, 1911, 2 days after critical drop in temperature. Patient still febrile.	Serum 119, Mar. 27, 1911, 4 days after critical drop in temperature. Patient still febrile.	Serum 119, Mar. 28, 1911, 5 days after critical drop in temperature. Patient still febrile.	Serum 119, Mar. 30, 1911, 7 days after critical drop in temperature. Patient afebrile.	Serum 119, Mar. 31, 1911, 8 days after critical drop in temperature. Patient afebrile.	Serum 119, Apr. 10, 1911, 18 days after critical drop in temperature. Patient afebrile.
0.1	0.2	—	—	—	D. 24 hrs.	D. 24 hrs.	D. 32 hrs.	D. 29 hrs.	D. 42 hrs.	D. 42 hrs.	D. 32 hrs.	D. 24 hrs.	—
0.01	0.2	—	—	—	D. 42 hrs.	D. 20 hrs.	D. 75 hrs.	D. 42 hrs.	D. 42 hrs.	D. 27 hrs.	—	D. 32 hrs.	—
0.001	0.2	D. 27 hrs.	D. 42 hrs.	D. 42 hrs.	D. 29 hrs.	D. 42 hrs.	D. 66 hrs.	Survived.	Survived.	Survived.	D. 42 hrs.	D. 42 hrs.	—
0.0001	0.2	D. 42 hrs.	D. 42 hrs.	D. 66 hrs.	D. 42 hrs.	D. 66 hrs.	Survived.	Survived.	D. 49 hrs.	D. 42 hrs.	D. 44 hrs.	D. 42 hrs.	D. 45 hrs.
0.00001	0.2	D. 50 hrs.	D. 42 hrs.	D. 32 hrs.	D. 42 hrs.	D. 42 hrs.	Survived.	Survived.	Survived.	D. 42 hrs.	D. 66 hrs.	D. 45 hrs.	D. 66 hrs.
0.000001	0.2	D. 45 hrs.	D. 43 hrs.	D. 42 hrs.	D. 47 hrs.	D. 66 hrs.	Survived.	Survived.	Survived.	Survived.	D. 45 hrs.	D. 96 hrs.	Survived.

Experiment 5.—The serum (132) was obtained from a patient with lobar pneumonia, terminating by lysis on from the third to the sixth day of the disease. The pneumococcus against which the specimens of sera were tested was isolated from the sputum, and the virulence raised by animal passage until 0.0001 c.c. was fatal for mice in forty-eight hours. The blood culture was negative.

Quantity of culture in c.c.	Quantity of serum in c.c.	Virulence; no serum.	Control serum, endocarditis.	Serum 132, Apr. 17, 1911, 1 day before drop in temperature.	Serum 132, Apr. 18, 1911, during falling temperature.	Serum 132, Apr. 20, 1911, 2 days after drop in temperature. Patient still febrile.	Serum 132, Apr. 23, 1911, 5 days after drop in temperature. Patient afebrile.
0.1	0.2	D. 30 hrs.	D. 30 hrs.	D. 41 hrs.	D. 30 hrs.	D. 41 hrs.	D. 41 hrs.
0.01	0.2	D. 41 hrs.	D. 41 hrs.	D. 30 hrs.	Survived.	Survived.	Survived.
0.001	0.2	D. 41 hrs.	Survived.	Survived.	Survived.	Survived.	Survived.
0.0001	0.2	D. 41 hrs.	Survived.	Survived.	Survived.	Survived.	Survived.
0.00001	0.2	Survived.	Survived.	Survived.	Survived.	Survived.	Survived.
0.000001	0.2	Survived.	Survived.	Survived.	Survived.	Survived.	Survived.

Experiment 6.—The patient from whom the serum (145) used in this experiment was obtained had typical lobar pneumonia. The temperature fell by lysis on the ninth day of the disease, rose again the next day, and remained slightly elevated until the fifteenth day, when it again returned to normal. The patient died thirty days later from a post-pneumonic nephritis. The organism was obtained from the sputum and was the so called *Pneumococcus mucosus.* It was of extremely high virulence for mice. The blood culture was negative.

Quantity of culture in c.c.	Quantity of serum in c.c.	Virulence; no serum.	Control serum, nephritis.	Serum 145, Apr. 5, 1911, 6 days before first normal temperature.	Serum 145, Apr. 8, 1911, 3 days before first normal temperature.	Serum 145, Apr. 11, 1911, day that temperature first reached normal.	Serum 145, Apr. 15, 1911, 4 days after first normal temperature: still slight fever.	Serum 145, Apr. 20, 1911, 9 days after first normal temperature. Patient afebrile.
0.1	0.2	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.
0.01	0.2	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.
0.001	0.2	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.	D. 21 hrs.	D. 45 hrs.	D. 21 hrs.	D. 21 hrs.
0.0001	0.2	D. 21 hrs.	D. 29 hrs.	D. 21 hrs.	D. 25 hrs.	Survived.	D. 21 hrs.	D. 25 hrs.
0.00001	0.2	D. 21 hrs.	D. 27 hrs.	D. 27 hrs.	D. 27 hrs.	D. 82 hrs.	D. 82 hrs.	D. 27 hrs.
0.000001	0.2	D. 27 hrs.	D. 82 hrs.	D. 29 hrs.	D. 46 hrs.	D. 82 hrs.	Survived.	D. 30 hrs.

Experiment 7.—The serum (411) for the following test was obtained from a patient, with severe lobar pneumonia, involving both lower lobes. The temperature fell by lysis on the seventh day of disease, reaching normal ten days later. The blood culture yielded *Diplococcus lanceolatus.* The organism, when isolated, was of low virulence for mice, 0.1 c.c. of a twenty-four hour broth culture failing to kill. Before testing the protective power of the patient's serum, the virulence was raised by animal passage until 0.000001 c.c. of a twenty-four hour broth culture was fatal for mice in forty-eight hours.

Quantity of culture in c.c.	Quantity of serum in c.c.	Virulence; no serum.	Control serum, syphilis.	Serum 411, Dec. 16, 1911, 3 days before beginning of lysis.	Serum 411, Dec. 19, 1911, during lysis.	Serum 411, Jan. 3, 1912, 14 days after temperature reached normal.
0.1	0.2	—	—	D. 18 hrs.	D. 18 hrs.	—
0.01	0.2	—	—	D. 18 hrs.	D. 24 hrs.	—
0.001	0.2	D. 36 hrs.	D. 36 hrs.	D. 18 hrs.	Survived.	D. 18 hrs.
0.0001	0.2	D. 5 days.	D. 36 hrs.	D. 18 hrs.	D. 54 hrs.	D. 4 days.
0.00001	0.2	D. 24 hrs.	D. 28 hrs.	D. 24 hrs.	D. 36 hrs.	Survived.
0.000001	0.2	D. 36 hrs.	D. 28 hrs.	D. 5 days.	Survived.	Survived.

Experiment 8.—The serum (503) for this experiment was obtained from a typical case of lobar pneumonia, terminating by crisis on the eighth day of the disease. The blood cultures during the course of the disease were negative. The protective power of the serum was tested against two strains of pneumococci; one, pneumococcus X, a stock culture, had received a great number of animal passages; the other, pneumococcus IX, had been isolated from the sputum of the patient and passed through five animals until 0.000001 c.c. of a twenty-four hour broth culture was fatal for mice in forty-eight hours. It was possible to protect mice against infection with both strains by the use of the serum of a horse immunized to a single strain of pneumococcus Neufeld I.

Protection of Mice against Pneumococcus X (Stock Organism).

Quantity of culture in c.c.	Quantity of serum in c.c.	Virulence; no serum.	Immune horse serum.	Serum 503, Jan. 25 1912, 5 days before crisis.	Serum 503, Jan. 29, 1912, 24 hours before crisis.	Serum 503, Jan. 30, 1912, day of crisis.	Serum 503, Feb. 14, 1912, 14 days after crisis.
0.1	0.2	—	Survived.	—	D. 20 hrs.	D. 22 hrs.	D. 28 hrs.
0.01	0.2	—	Survived.	—	D. 24 hrs.	D. 28 hrs.	D. 23 hrs.
0.001	0.2	D. 36 hrs.	Survived.	D. 24 hrs.	D. 28 hrs.	D. 36 hrs.	D. 36 hrs.
0.0001	0.2	D. 36 hrs.	Survived.	D. 36 hrs.	D. 36 hrs.	D. 46 hrs.	D. 50 hrs.
0.00001	0.2	D. 36 hrs.	Survived.	D. 36 hrs.	D. 46 hrs.	D. 36 hrs.	D. 50 hrs.
0.000001	0.2	D. 23 hrs.	Survived.	D. 36 hrs.	D. 60 hrs.	D. 36 hrs.	D. 36 hrs.

Protection of Mice against Pneumococcus IX (Organism from Sputum of Patient 503).

Quantity of culture in c.c.	Quantity of serum in c.c.	Virulence; no serum.	Immune horse serum.	Serum 503, Jan. 25, 1912, 5 days before crisis.	Serum 503, Jan. 29, 1912, 24 hours before crisis.	Serum 503, Jan. 30, 1912, day of crisis.	Serum 503, Feb. 14, 1912, 14 days after crisis.
0.1	0.2	—	—	—	—	—	—
0.01	0.2	—	—	—	D. 30 hrs.	D. 30 hrs.	—
0.001	0.2	D. 48 hrs.	Survived.	D. 30 hrs.	D. 46 hrs.	Survived.	Survived.
0.0001	0.2	D. 30 hrs.	Survived.	D. 48 hrs.	D. 46 hrs.	Survived.	Survived.
0.00001	0.2	D. 46 hrs.	Survived.	D. 48 hrs.	Survived.	Survived.	Survived.
0.000001	0.2	D. 49 hrs.	Survived.	D. 46 hrs.	D. 46 hrs.	Survived.	Survived.

Experiment 9.—The following specimens of sera (501) were obtained from a patient with typical pneumonia of the right upper lobe. There was a pseudo-crisis on the fifth day of the disease, and a true crisis on the following day, after which the temperature remained normal. The pneumococcus used in the tests was isolated from the patient's sputum and the virulence raised by animal passage until 0.000001 c.c. of a twenty-four hour broth culture was fatal in thirty-six hours. The blood culture was negative throughout the disease.

Quantity of culture in c.c.	Quantity of serum in c.c.	Virulence; no serum.	Control serum, leg ulcer.	Serum 501, Feb. 19, 1912, 3 days before crisis.	Serum 501, Feb. 21, 1912, 1 day before crisis. Day of pseudocrisis.	Serum 501, Feb. 23, 1912, 1 day after crisis.	Serum 501, Mar. 11, 1912, 16 days after crisis.
0.1	0.2	—	—	—	D. 18 hrs.	D. 18 hrs.	D. 18 hrs.
0.01	0.2	—	—	—	D. 18 hrs.	D. 18 hrs.	D. 18 hrs.
0.001	0.2	D. 36 hrs.	D. 36 hrs.	D. 18 hrs.	D. 36 hrs.	D. 19 hrs.	D. 36 hrs.
0.0001	0.2	D. 36 hrs.	D. 36 hrs.	D. 36 hrs.	D. 36 hrs.	D. 36 hrs.	Survived.
0.00001	0.2	D. 36 hrs.	D. 36 hrs.	D. 36 hrs.	D. 36 hrs.	D. 48 hrs.	Survived.
0.000001	0.2	D. 36 hrs.	D. 36 hrs.	D. 36 hrs.	D. 36 hrs.	D. 36 hrs.	Survived.

Experiment 10.—The serum (502) for this test was obtained from a patient suffering from a severe attack of lobar pneumonia. The temperature fell by lysis on the eighth day of the disease, reaching normal on the tenth day. The blood cultures were negative. The pneumococcus used for infection of animals was obtained from the patient's sputum, and after several animal passages 0.000001 c.c. of a twenty-four hour broth culture was fatal for mice in thirty-six hours.

Quantity of culture in c.c.	Quantity of serum in c.c.	Virulence; no serum.	Control serum, leg ulcer.	Serum 502, Feb. 19, 1912, 6 days before drop in temperature.	Serum 502, Feb. 21, 1912, 3 days before drop in temperature.	Serum 502, Feb. 27, 1912, 1st day of normal temperature.	Serum 502, Mar. 11, 1912, 13 days after normal temperature
0.1	0.2	—	—	—	—	D. 18 hrs.	D. 18 hrs.
0.01	0.2	—	—	—	—	D. 30 hrs.	D. 18 hrs.
0.001	0.2	—	—	—	—	D. 18 hrs.	D. 30 hrs.
0.0001	0.2	D. 36 hrs.	D. 18 hrs.	D. 22 hrs.	D. 36 hrs.	D. 24 hrs.	D. 22 hrs.
0.00001	0.2	D. 24 hrs.	D. 24 hrs.	D. 36 hrs.	D. 36 hrs.	D. 36 hrs.	D. 18 hrs.
0.000001	0.2	D. 36 hrs.	D. 36 hrs.	D. 36 hrs.	D. 36 hrs.	D. 36 hrs.	D. 36 hrs.

Experiment 11.—The serum (554) used in this experiment came from a patient with typical pneumonia of the left lower lobe. The temperature fall was by crisis on the sixth day of the disease. The blood culture was negative. The pneumococcus was isolated from the sputum and the virulence raised until 0.000001 c.c. was fatal for mice in thirty-six hours.

Quantity of culture in c.c.	Quantity of serum in c.c.	Virulence; no serum.	Control serum, leg ulcer.	Serum 554, Mar. 16, 1912, 1 day before crisis.	Serum 554, Mar. 18, 1912, 12 hours after crisis.	Serum 554, Apr. 5, 1912, 17 days after crisis.
0.1	0.2	—	—	—	D. 18 hrs.	D. 18 hrs.
0.01	0.2	—	—	—	D. 28 hrs.	D. 18 hrs.
0.001	0.2	D. 28 hrs.	D. 18 hrs.	D. 18 hrs.	D. 3 days.	D. 3 days.
0.0001	0.2	D. 28 hrs.	D. 18 hrs.	D. 18 hrs.	D. 6 days.	Survived.
0.00001	0.2	D. 18 hrs.	Survived.	D. 28 hrs.	D. 3 days.	Survived.
0.000001	0.2	D. 36 hrs.	Survived.	D. 36 hrs.	Survived.	Survived.

Experiment 12.—The serum (538) for this experiment was obtained from a patient with a moderately severe attack of lobar pneumonia. The temperature fell by crisis on the seventh day of the disease. The blood cultures were negative. The organism used was a pneumococcus obtained from the patient's sputum. The virulence after animal passage was high, and 0.000001 c.c. of a twenty-four hour broth culture was fatal for mice in thirty-six hours.

Quantity of culture in c.c.	Quantity of serum in c.c.	Virulence; no serum.	Control serum, syphilis.	Serum 538, Mar. 18, 1912, 1 day before crisis.	Serum 538, Mar. 21, 1912, 36 hours after crisis.	Serum 538, Apr. 5, 1912, 16 days after crisis.
0.1	0.2	—	—	—	D. 18 hrs.	D. 18 hrs.
0.01	0.2	—	—	—	D. 24 hrs.	D. 18 hrs.
0.001	0.2	—	—	—	D. 24 hrs.	D. 24 hrs.
0.0001	0.2	D. 24 hrs.	D. 24 hrs.	D. 24 hrs.	Survived.	D. 24 hrs.
0.00001	0.2	D. 36 hrs.	D. 24 hrs.	D. 24 hrs.	Survived.	D. 24 hrs.
0.000001	0.2	D. 36 hrs.	D. 24 hrs.	D. 36 hrs.	Survived.	D. 36 hrs.

Experiment 13.—The serum (606) for this experiment was obtained from a patient with a severe attack of lobar pneumonia. The temperature fell by lysis on the eleventh day of the disease, but remained slightly elevated until the seventeenth day. The physical signs in the lungs cleared up slowly. The blood culture showed pneumococcus on the seventh day, and the organisms disappeared from the blood on the tenth day. The pneumococcus, when isolated, killed mice in the higher dilutions in from three to four days.

Quantity of culture in c.c.	Quantity of serum in c.c.	Virulence; no serum.	Control serum, syphilis.	Serum 606, Apr. 30, 1912, 4 days before beginning of lysis.	Serum 606, May 1, 1912, 3 days before beginning of lysis.	Serum 606, May 3, 1912, 1 day before beginning of lysis.	Serum 606, May 6, 1912, during lysis.	Serum 606, May 10, 1912, 1st day of normal temperature.	Serum 606, May 20, 1912, 10 days after normal temperature.
0.1	0.2	—	D. 40 hrs.	D. 26 hrs.	D. 24 hrs.	D. 24 hrs.	D. 20 hrs.	D. 20 hrs.	D. 48 hrs.
0.01	0.2	D. 42 hrs.	D. 30 hrs.	D. 40 hrs.	D. 26 hrs.	D. 20 hrs.	D. 26 hrs.	D. 48 hrs.	Survived.
0.001	0.2	D. 42 hrs.	D. 24 hrs.	D. 40 hrs.	D. 40 hrs.	D. 40 hrs.	D. 72 hrs.	D. 30 hrs.	Survived.
0.0001	0.2	D. 4 days.	D. 72 hrs.	D. 4 days.	D. 40 hrs.	D. 4 days.	D. 48 hrs.	D. 48 hrs.	Survived.
0.00001	0.2	D. 4 days.	D. 4 days.	D. 40 hrs.	D. 40 hrs.	D. 72 hrs.	D. 80 hrs.	Survived.	D. 3 days.
0.000001	0.2	D. 48 hrs.	D. 40 hrs.	D. 48 hrs.	D. 72 hrs.	D. 30 hrs.	D. 4 days.	D. 4 days.	Survived.

Experiment 14.—The serum (553) for the following test was obtained from a patient with a moderately severe pneumonia of the left lower lobe. There was a pseudocrisis on the fourth day of the disease. The true crisis occurred on the eighth day of the disease. The blood cultures were negative. The virulence of the pneumococcus isolated from the patient's sputum could not be raised successfully, so the same organism was used for infection of animals as in experiment 13.

Quantity of culture in c.c.	Quantity of serum in c.c.	Virulence; no serum.	Control serum, syphilis.	Serum 553, Mar. 5, 1912, 6 days before crisis.	Serum 553, Mar. 7, 1912, 4 days before crisis. Day of pseudo-crisis.	Serum 553, Mar. 11, 1912, day of crisis.	Serum 553, Mar. 26, 1912, 15 days after crisis.
0.1	0.2	—	D. 40 hrs.	—	—	D. 24 hrs.	D. 40 hrs.
0.01	0.2	D. 42 hrs.	D. 30 hrs.	—	—	D. 40 hrs.	D. 20 hrs.
0.001	0.2	D. 42 hrs.	D. 24 hrs.	D. 4 days.	D. 40 hrs.	D. 40 hrs.	D. 5 days.
0.0001	0.2	D. 4 days.	D. 72 hrs.	D. 4 days.	D. 4½ days.	Survived.	D. 40 hrs.
0.00001	0.2	D. 4 days.	D. 4 days.	D. 4½ days.	D. 4 days.	Survived.	Survived.
0.000001	0.2	D. 48 hrs.	D. 4 days.	D. 4 days.	Survived.	Survived.	Survived.

DISCUSSION.

Specimens of sera obtained at various stages of the disease in fourteen patients have been studied. The specimens of sera from ten of these patients were tested against homologous organisms. The sera from three of the cases were tested against a stock organism, Neufeld I, and from one case against an organism isolated from the blood of patient 606. The appearance of protective substances is more evident when sera are tested against homologous organisms. In the ten groups of sera tested against homologous organisms, all but one exhibited at some time the power to protect mice against infection. Of the four groups of sera tested against the stock organisms, three showed at no time any protective power. In experiment 14, the serum obtained on the day of crisis protected against 100 lethal doses of an organism obtained from the blood of patient 606. It has been shown by Neufeld and Haendel[7] that the immune bodies for various races of typical pneumococci are highly specific, and we have also found this to be the case in our study of the protective power of an immune serum produced from a type strain. It is, therefore, evident that in the present study the results can only be of importance in the cases where homologous organisms were employed. This is shown by experiment 8, in which the same specimens of sera were tested against two strains of pneumococci,

[7] Neufeld, F., and Haendel, *Arb. a. d. k. Gsndhtsamte,* 1910, xxxiv, 293.

one a stock culture and the other an organism isolated from the patient's sputum. At no time did the serum protect against the stock strain, whereas the post-critical sera protected regularly against one thousand times the lethal dose of the homologous organism.

The amount of protection afforded by the pneumonic sera examined has varied in different individuals. In comparison with the potency of sera obtainable by active immunization of larger animals, it has been small. The greatest amount of protection against strains of maximum virulence is seen in experiments 4 and 8. In both these instances it was possible to protect mice with specimens of serum obtained after the crisis against 0.001 of a cubic centimeter of a broth culture of a highly virulent pneumococcus, of which 0.000001 of a cubic centimeter of the same culture was fatal for mice in forty-eight hours, thus affording protection against one thousand times the lethal dose, a value that corresponds favorably with that of some of the commercial immune sera. In other experiments the protective power of the sera tested varied from ten to one hundred times the lethal dose.

The time at which the appearance of immune bodies in the blood was demonstrable varied somewhat. In only four cases was there any protective power demonstrable in the serum obtained before the crisis. In experiment 1 protective substances were present twelve hours before the drop in temperature. Inasmuch as no earlier specimen of serum had been obtained in this instance, it is impossible to say how long these bodies had been present. In experiment 5 serum obtained two days before the temperature fell showed slight protective power, but no greater than control serum from a patient with chronic endocarditis. In two other experiments, 8 and 14, single animals that had received ante-critical sera survived. In seven cases protective substances either appeared for the first time or showed a marked increase in amount at the time of crisis or, in case of lysis, during the period when the symptoms were abating. In some of these instances no specimens of serum were obtained immediately before the crises, so that the immune bodies may have been present twenty-four hours or longer before the disappearance of symptoms. However, in experiment 4 the

specimen of serum obtained twenty hours before the beginning of the critical drop in temperature contained no protective bodies. In two cases the serum taken during the period of defervescence exhibited little or no power of protection, even against homologous strains of pneumococcus, and it was not for some time later, in one case sixteen days, that the presence of protective substances in the blood was demonstrated. The sera of seven patients showed undiminished protective power as long as the individuals were under observation,—from five to twenty days. In five cases there was a drop in the protective power during the period of convalescence. This diminution varied from a slight loss to complete absence of protective substances. In several instances there was a slight return of protective power during late convalescence. It was thought at one time that the disappearance of antibodies might have some connection with the continuance of slight fever after the critical fall of temperature, but the same phenomenon has been observed in patients that remained afebrile after the crisis.

CONCLUSIONS.

These experiments demonstrate that protective substances are usually present in the blood of patients recovering from lobar pneumonia. As a rule, the appearance of protective bodies in the blood, when demonstrable, coincides rather sharply with the period of critical fall in temperature and the disappearance of symptoms. These substances are not present in the blood in any measurable degree before the crisis, but afterward they may reach a considerable concentration. In certain instances, protective substances either become evident some time after the occurrence of the crisis, or cannot be demonstrated at any period of the disease. Experiments in which it is possible to test serum against an homologous strain of pneumococcus yield in the majority of cases evidence of the presence of protective bodies; whereas in those in which stock cultures are used, the serum, as a rule, shows no protection. The development of specific protective substances in the serum of patients with lobar pneumonia suggests that these bodies may play a part in the mechanism of recovery.

THE OCCURRENCE AND VIRULENCE OF PNEUMOCOCCI IN THE CIRCULATING BLOOD DURING LOBAR PNEUMONIA AND THE SUSCEPTIBILITY OF PNEUMOCOCCUS STRAINS TO UNIVALENT ANTIPNEUMOCOCCUS SERUM.*

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The suggestion has been made that lobar pneumonia is primarily a blood infection and the local process in the lung is but part of a generalized condition. This view arose from the discovery that the pneumococcus may at times appear in the circulating blood of persons suffering from pneumonia. Reports in recent years have varied considerably as to the frequency with which it is possible to obtain the pneumococcus from the blood. Some observers have obtained positive cultures in from 20 to 40 per cent. of cases examined and, as they were the more severe cases, they think the presence of the pneumococcus in the blood is of bad prognostic import. Others, claiming a more suitable technique, have found the pneumococcus in the blood during pneumonia with great constancy, and attach very little prognostic significance to its presence. It has been stated that the organisms may be present in considerable numbers, especially in fatal cases, and Fränkel[1] mentions a case in which cultures from the blood during life yielded 2,700 colonies per cubic centimeter of blood.

In thirty-seven cases of lobar pneumonia studied in this hospital, the pneumococcus was not found constantly present in the blood. In those cases in which a positive culture was obtained, the course of the infection was invariably severe and in most instances terminated fatally. Studies of inflammation have shown that micro-

* Received for publication, August 1, 1912.

[1] Fränkel, A. A., Internationale Beiträge zu innere Medizin, Berlin, 1902, ii, 103.

organisms in the early stages of the process are taken up by the lymphatics and are passed through them to the adjacent lymph glands. As the inflammatory process progresses, the lymph glands swell, the lymphatic sinuses become packed with cells, and the gland in this way functions as a bacterial filter. That pneumococci may pass the barrier of lymphatic glands in the early stages of pneumonia and in this manner reach the circulation is not unlikely. The majority, however, are probably destroyed rapidly and never develop sufficiently to be readily demonstrable in cultures from the blood. Some, having gained access, may grow slowly for a time and then disappear, or as the body defenses become weakened by intoxication, they may multiply rapidly and cause the death of the individual. In certain cases of pneumonia, it seems that the pneumococci either invade the blood in large numbers or else, having arrived there, grow so actively that death ensues from septicemia before any considerable area of lung has become involved. The possibility must be borne in mind that invasion of the blood may not occur by way of lymphatic channels alone, but that vessel walls may become so injured by bacterial products that direct extension of the infection through them into the blood stream arises. However the organisms may get into the blood, the important question is whether, once having gotten there, they are able to develop. As long as the number of pneumococci in the blood is inconsiderable, probably no great harm results, but as soon as they begin to grow actively, the condition is a far more serious menace to the life of the individual than the local process in the lung.

CASES STUDIED.

In the following cases blood was obtained for the culture by venous puncture. Usually about 5 cubic centimeters of blood were added to a flask containing from 75 to 125 cubic centimeters of bouillon prepared from fresh meat, to which from 20 to 25 cubic centimeters of normal sodium hydrate per liter had been added. Pneumococcus grows luxuriantly and without difficulty in this medium. Repeated cultures were made from the same individual in many cases and in some instances solid cultures were prepared in

order to determine the number of pneumococci per cubic centimeter of blood.

Among the thirty-seven cases detailed in table I, pneumococci were present in the blood at some stage of the disease in eighteen instances, approximately in 50 per cent. of the cases. Of these eighteen cases, fourteen, or 77 per cent., were fatal, whereas four cases recovered. Of the nineteen cases in which blood cultures were negative, fifteen, or 79 per cent., recovered and four died. The patients were chiefly persons of a low social class, and many of them were habitual users of alcohol. The total mortality was unusually high, 48 per cent., and of the total number of fatal cases the pneumococcus was isolated from the blood in 77 per cent. In four instances the first blood culture was negative and subsequent cultures at a later stage of the disease showed the presence of the pneumococcus. In the majority of instances, where a positive culture was obtained, the number of bacteria per cubic centimeter of blood was estimated from solid cultures. The counts, as a rule, were extraordinarily high shortly before or at the time of death, showing thousands of colonies. The extent of the local disease was not always comparable to the severity of blood invasion, and in two instances seemed inconspicuous. In two fatal cases where low bacterial counts were obtained from the blood, the local process in the lung spread with great rapidity. In case XV there were 10,000 pneumococci per cubic centimeter of blood on the fourth day of the disease. The highest count obtained was in case XXXVII, being 65,000 colonies per cubic centimeter of blood obtained at the time of death on the eighth day of disease. Of the four fatal cases without positive blood cultures, one died of nephritis after the subsidence of the pneumonia, and three showed a spreading infection in the lungs. Of the nineteen cases that recovered, four had pneumococci in the blood, and in fifteen the blood cultures were negative. The course of the disease in the four cases with positive blood cultures was severe in three instances and moderate in one. In two of the cases the pneumococcus isolated from the blood was avirulent, in one it killed animals slowly, and in the other was of high virulence. In recovered cases XXXIV and XXXVI, successive counts of the bacteria in the blood were made. The maximum count in case

TABLE I.

Number of case.	Number of record.	Age.	Day of disease.	Time before death.	Medium.	Result.	Recovery or death.	Day of disease.	Remarks.
I	109	31 yrs.	4th	—	Bouillon flask	Negative	Recovered (crisis)	4th	Mild infection involving part of the left lower lobe. Convalescence normal.
II	123	34 yrs.	7th	—	Bouillon flask	Negative	Recovered (lysis)	7th	Moderate infection of right lower lobe. Convalescence normal.
III	116	11 yrs.	?	—	Bouillon flask	Negative	Recovered (crisis)	5th	Mild infection with indefinite localizing signs in the right lower lobe.
IV	145	54 yrs.	3d	42 dys.	Bouillon flask,	Negative	Died	45th	Severe infection involving the left lower lobe. Patient died of post-pneumonic nephritis on the 45th day after the onset of pneumonia.
			9th	36 dys.	bouillon flask	Negative			
V	132	32 yrs.	5th	—	Bouillon flask	Negative	Recovered (lysis)	4th	Mild infection involving the left lower lobe. No complication. Culture made during lysis.
VI	405	24 yrs.	8th	—	Bouillon flask	Negative	Recovered (lysis)	8th	Mild infection involving a portion of the left lower lobe. Convalescence uncomplicated.
VII	411	28 yrs.	7th	—	Bouillon flask	Positive	Recovered (lysis)	9th	A very severe infection involving both lower lobes. Convalescence normal.
VIII	418	26 yrs.	2d	—	Bouillon flask	Negative	Recovered (lysis)	4th	A mild infection involving the left lower lobe. Convalescence normal.
IX	419	47 yrs.	10th	—	Bouillon flask	Negative	Recovered (lysis)	11th	A very severe infection involving both lower lobes. Convalescence uncomplicated.
X	413	24 yrs.	3d	—	Bouillon flask	Negative	Died	4th	A severe, rapidly spreading infection involving successively the left lower, left upper, and right lower lobes. Post-operative pneumonia.
XI	428	32 yrs.	4th	3 dys.	Bouillon flask,	Positive	Died	7th	A severe infection involving the left lower lobe. At autopsy, seven hours after death, the blood contained about 15,000 pneumococci per cubic centimeter.
			Autopsy	—	sheep serum, glucose agar	Positive			
XII	423		5th	2 dys.	Bouillon flask,	Positive	Died	7th	A very severe infection involving the whole right lung.
					sheep serum, glucose agar	Positive			
XIII	454	23 yrs.	3d	—	Bouillon flask	Negative	Recovered (crisis)	3d	Mild infection without frank consolidation.
XIV	439	31 yrs.	6th		Bouillon flask,	Negative	Died	10th	A very severe infection involving first the right lower lobe and extending to the right middle and upper lobes on the ninth day of the disease.
			9th		bouillon flask,	Negative			
			Autopsy		sheep serum, glucose agar	Negative			
XV	438	49 yrs.	2d	2 dys.	Bouillon flask,	Positive	Died	4th	Infection very severe. Lung signs not conspicuous. Partial consolidation of the left lower lobe on the second day, and beginning involvement of the right lower on the fourth day. Ten hours before death the blood contained 10,000 pneumococci per c.c.
			4th	10 hrs.	sheep serum, glucose agar	Positive	—		
XVI	503	32 yrs.	3d	—	Bouillon flask	Negative	Recovered (crisis)	7th	Patient's condition good throughout course of disease. Developed serofibrinous pleurisy during convalescence. Cultures from pleural fluid sterile.
XVII	453	71 yrs.	Autopsy	—	Sheep serum, glucose agar	Positive	Died	5th	Severe infection involving entire right lung. Blood at autopsy twenty hours after death

					flask		(crisis)		of disease. Developed serofibrinous pleurisy during convalescence. Cultures from pleural fluid sterile.
XVII	453	71 yrs.	Autopsy	—	Sheep serum, glucose agar	Positive	Died	5th	Severe infection involving entire right lung. Blood at autopsy twenty hours after death contained about 50,000 pneumococci per c.c.
XVIII	460	37 yrs.	3d	3 dys.	Bouillon flask,	Negative	Died	6th	The infection was a very severe one, involving first the lower right lobe and finally extending to the right middle and upper lobes, and involving the posterior two thirds of the left lower lobe. The cultures at autopsy were from the heart's blood.
			5th	1 dy.	bouillon flask,	Negative	—		
			Autopsy	—	sheep serum, glucose agar	Negative	—		
XIX	484	53 yrs.	3d	5 dys.	Bouillon flask,	Negative	Died	8th	Very severe, spreading infection involving first the right lower, then right middle lobes and extending on the sixth day to the left lower lobe. The organism isolated from the blood was *Pneumococcus mucosus*.
			7th	1 dy.	bouillon flask	Positive	—		
XX	472	32 yrs.	3d	3 dys.	Bouillon flask,	Negative	Died	6th	Patient admitted with involvement of left lower lobe. The condition remained good until the fifth day, the time of the first positive blood culture. Condition then became rapidly worse, death occurring thirty hours later. The number of pneumococci in the blood a few hours before death was estimated at more than 1,000 per c.c.
			5th	24 hrs.	bouillon flask,	Positive	—		
			6th	2 hrs.	bouillon flask	Positive	—		
XXI	497	17 yrs.	5th	—	Bouillon flask	Negative	Recovered (lysis)	7th	Moderately severe infection, involving left lower lobe. Convalescence uncomplicated.
XXII	502	28 yrs.	3d	—	Bouillon flask,	Negative	Recovered (lysis)	8th	Course of disease severe. Infection involved apparently only right middle lobe and seemed to spread throughout this lobe slowly. Convalescence uncomplicated.
			5th	—	bouillon flask	Negative			
XXIII	501	22 yrs.	3d	—	Bouillon flask,	Negative	Recovered (crisis)	6th	Moderately severe infection, involving the right upper lobe. Pseudocrisis on the fifth day. Convalescence normal.
			6th	—	bouillon flask	Negative			
XXIV	480	42 yrs.	6th	6 hrs.	Bouillon flask,	Positive	Died	6th	Severe infection involving right lower and middle lobes. Blood six hours before death contained 15,000 colonies of pneumococcus per c.c.
					sheep serum, glucose agar	Positive			
XXV	487	37 yrs.	3d	4 dys.	Bouillon flask,	Positive	Died	7th	Very severe, spreading infection, involving first the right lower and middle lobes, and extending on the fifth day throughout the left lower lobe. The blood at the time of death contained 5,000 colonies per c.c.
			7th	At time of death	sheep serum, glucose agar	Positive	—		
XXVI	533	53 yrs.	6th	6 dys.	Bouillon flask,	Positive	Died	12th	The lesion in the lungs declared itself slowly, complete consolidation of the right upper and middle lobes, with some signs in the right lower lobe only becoming definite on the eleventh day of the disease. A bouillon culture on the sixth day of disease yielded pure pneumococcus. Plates on the eighth day showed 2,900 colonies of mixed pneumococcus and *Staphylococcus albus* per c.c. of blood. On the tenth day there were 245 colonies of pure pneumococcus, and on the eleventh day 15,000 colonies per c.c. of blood.
			8th	4 dys.	glucose agar	Positive	—		
			10th	2 dys.	glucose agar,	Positive	—		
			11th	18 hrs.	glucose agar	Positive	—		

			4th	3 dys.	Bouillon flask,	Positive	Died	7th	A very severe infection involving first the left lower lobe, spreading rapidly from the fifth to the sixth day to the left upper, right lower, and right upper lobes. Degree of involvement determined at autopsy. On the seventh day of disease, plates yielded 125 colonies of pneumococcus per c.c. of blood.
			7th	At time of death	glucose agar	Positive	—		
XXVIII	538	22 yrs.	4th	—	Bouillon flask,	Negative	Recovered (crisis)	7th	Severe infection first involving the left lower lobe, extending on the sixth day throughout a large part of the left upper lobe. Convalescence uncomplicated.
			6th	—	bouillon flask	Negative			
XXIX	554	20 yrs.	5th	—	Bouillon flask	Negative	Recovered (crisis)	6th	Moderate infection involving left lower lobe. No complications.
XXX	119	46 yrs.	7th	—	Bouillon flask	Positive	Recovered (lysis)	10th	Moderately severe infection involving the right lower lobe. Convalescence uncomplicated.
XXXI	525	35 yrs.	4th	2 dys.	Bouillon flask,	Positive	Died	6th	Very severe infection involving the right upper and middle lobes. Plates on the fifth day of the disease yielded 4,500 colonies of pneumococcus per c.c. of blood.
			5th	15 hrs.	glucose agar	Positive	—		
XXXII	553	21 yrs.	3d	—	Bouillon flask,	Negative	Recovered (crisis)	8th	Moderately severe infection involving the left lower lobe. Delayed resolution.
			5th	—	bouillon flask	Negative			
XXXIII	544	19 yrs.	4th	7 dys.	Bouillon flask,	Negative	Died	11th	A very severe infection complicated by secondary syphilis. The local infection in the lungs spread progressively, involving first the right lower lobe, then the left lower, and lastly the left upper. On the eleventh day of the disease plates yielded 2,500 colonies of pneumococcus per c.c. of blood. Organism isolated from blood was *Pneumococcus mucosus*.
			8th	3 dys.	bouillon flask,	Negative	—		
			11th	1 hr.	glucose agar	Positive	—		
XXXIV	551	50 yrs.	3d	—	Bouillon flask,	Positive	Recovered (lysis)	7th	Severe infection involving left upper and lower lobes. The temperature fell by lysis and empyema developed. Glucose agar plates showed 1½ colonies of pneumococcus per c.c. of blood on the third day of disease, 60 colonies on the fifth day, and less than 1 colony on the sixth day. On the seventh day a bouillon flask was negative. On the twelfth day thoracentesis yielded purulent fluid containing many encapsulated pneumococci.
					glucose agar,	Positive			
			5th	—	glucose agar,	Positive	—		
			6th	—	glucose agar,	Positive	—		
			7th	—	bouillon flask	Negative	—		
XXXV	547	33 yrs.	3d	7 dys.	Bouillon flask,	Negative	Died	10th	Very severe infection involving first the right lower lobe, spreading to right middle and upper lobes on the seventh day of the disease, and to the left lower on the eighth day. On the seventh day plates showed 4 colonies of pneumococcus per c.c. of blood, and on the ninth day 21 colonies.
			7th	48 hrs.	bouillon flask,	Positive	—		
					glucose agar,	Positive	—		
			9th	18 hrs.	glucose agar	Positive	—		

									mococcus and *Staphylococcus albus* per c.c. of blood. On the tenth day there were 245 colonies of pure pneumococcus, and on the eleventh day 15,000 colonies per c.c. of blood.
XXVII	535	34 yrs.	4th	3 dys.	Bouillon flask,	Positive	Died	7th	A very severe infection involving first the left lower lobe, spreading rapidly from the fifth to the sixth day to the left upper, right lower, and right upper lobes. Degree of involvement determined at autopsy. On the seventh day of disease, plates yielded 125 colonies of pneumococcus per c.c. of blood.
			7th	At time of death	glucose agar	Positive	—		
XXVIII	538	22 yrs.	4th	—	Bouillon flask,	Negative	Recovered (crisis)	7th	Severe infection first involving the left lower lobe, extending on the sixth day throughout a large part of the left upper lobe. Convalescence uncomplicated.
			6th	—	bouillon flask	Negative			
XXIX	554	20 yrs.	5th	—	Bouillon flask	Negative	Recovered (crisis)	6th	Moderate infection involving left lower lobe. No complications.
XXX	119	46 yrs.	7th	—	Bouillon flask	Positive	Recovered (lysis)	10th	Moderately severe infection involving the right lower lobe. Convalescence uncomplicated.
XXXI	525	35 yrs.	4th	2 dys.	Bouillon flask,	Positive	Died	6th	Very severe infection involving the right upper and middle lobes. Plates on the fifth day of the disease yielded 4,500 colonies of pneumococcus per c.c. of blood.
			5th	15 hrs.	glucose agar	Positive	—		
XXXII	553	21 yrs.	3d	—	Bouillon flask,	Negative	Recovered (crisis)	8th	Moderately severe infection involving the left lower lobe. Delayed resolution.
			5th	—	bouillon flask	Negative			
XXXIII	544	19 yrs.	4th	7 dys.	Bouillon flask,	Negative	Died	11th	A very severe infection complicated by secondary syphilis. The local infection in the lungs spread progressively, involving first the right lower lobe, then the left lower, and lastly the left upper. On the eleventh day of the disease plates yielded 2,500 colonies of pneumococcus per c.c. of blood. Organism isolated from blood was *Pneumococcus mucosus*.
			8th	3 dys.	bouillon flask,	Negative	—		
			11th	1 hr.	glucose agar	Positive	—		
XXXIV	551	50 yrs.	3d	—	Bouillon flask,	Positive	Recovered (lysis)	7th	Severe infection involving left upper and lower lobes. The temperature fell by lysis and empyema developed. Glucose agar plates showed 1½ colonies of pneumococcus per c.c. of blood on the third day of disease, 60 colonies on the fifth day, and less than 1 colony on the sixth day. On the seventh day a bouillon flask was negative. On the twelfth day thoracentesis yielded purulent fluid containing many encapsulated pneumococci.
					glucose agar,	Positive			
			5th	—	glucose agar,	Positive	—		
			6th	—	glucose agar,	Positive	—		
			7th	—	bouillon flask	Negative	—		
XXXV	547	33 yrs.	3d	7 dys.	Bouillon flask,	Negative	Died	10th	Very severe infection involving first the right lower lobe, spreading to right middle and upper lobes on the seventh day of the disease, and to the left lower on the eighth day. On the seventh day plates showed 4 colonies of pneumococcus per c.c. of blood, and on the ninth day 21 colonies.
			7th	48 hrs.	bouillon flask,	Positive	—		
					glucose agar,	Positive	—		
			9th	18 hrs.	glucose agar	Positive	—		
XXXVI	606	30 yrs.	7th	—	Bouillon flask,	Positive	Recovered (lysis)	10th	A severe infection involving first the right upper and middle lobes, extending on the ninth day to the right lower lobe. Plates on the eighth day of the disease yielded less than
			8th	—	glucose agar,	Positive			

			10th	—	glucose agar,	Negative			Convalescence uncomplicated.
			12th	—	bouillon flask	Negative	—		
XXXVII	617	37 yrs.	6th	2 dys.	Bouillon flask,	Positive	Died	8th	A very severe infection involving the right lower and middle lobes. Plates on the seventh day of the disease showed 3,000 colonies of pneumococcus per c.c. of blood. At the time of death the blood contained 65,000 pneumococci per c.c.
			7th	24 hrs.	glucose agar,	Positive	—		
			At time of death	—	sheep serum, glucose agar	Positive	—		

XXXIV was sixty colonies per cubic centimeter on the fifth day of the disease. On the sixth day the count had dropped to less than one colony per cubic centimeter, and on the seventh day, the day of lysis, the culture was negative. The organism isolated in this case was highly virulent and the course of the disease was complicated by empyema. In case XXXVI the maximum count per cubic centimeter of blood was less than one colony on the eighth day of the disease. On the tenth day, the day of lysis, the blood culture was negative. Of the fifteen recovered cases with negative blood cultures, the course of the disease was usually not very severe and there seemed to be little tendency for the disease to spread beyond the limits of the lobe of lung originally infected.

The object of this study has been to emphasize the infectious side of lobar pneumonia. The two most striking characteristics of pneumococcus infection of the lung are the tendency of the local lesion to spread and the tendency to serious invasion of the blood by the bacteria. The local process usually develops until it meets the opposition to further extension afforded by the pleural membrane. The opinion is quite generally prevalent among clinicians that the extent of involvement of lung tissue in pneumonia bears no relation to the severity of the disease. In support of this assumption, severe and rapidly fatal cases are cited in which simply one upper lobe is involved, or in which the pneumonic process lies deep within a lobe and is difficult to recognize clinically. The cause of death is ascribed to failure of the circulation dependent upon the unusually severe intoxication attending such infections. Careful study of the blood in these instances would probably reveal a septicemic condition sufficient to account for the fatal result, regardless of the local disease in the lung. Considerable doubt exists as to whether any previously strong individual dies from the intoxication arising from a small area of pneumonic consolidation of the lung. Certainly in this hospital no instance of death occurred when the process was confined to a single lobe and where there was no bacterial invasion of the blood. Death in pneumonia seems therefore to depend chiefly upon an extensive invasion of the blood by the organisms, or upon a rapid and extensive spread of the local lesion in the lung.

VIRULENCE FOR WHITE MICE OF PNEUMOCOCCI ISOLATED FROM THE BLOOD DURING PNEUMONIA.

Frequent attempts have been made to correlate the progress of lobar pneumonia with variations in the virulence of the pneumococcus during the course of the infection. A satisfactory assumption would seem to be that the more virulent the pneumococcus concerned, the more severe the character of the disease. Aside from the fact that virulence must be tested upon a different species of animal, the varying efficiency of the resources of the host, in the struggle for mastery between parasite and host, makes it impossible to predict the outcome of an infection from a knowledge of the virulence alone. Studies in the variation of virulence of the pneumococcus have been made largely upon organisms obtained from the sputum. In general, the results have been variable, but there seems to be no definite relationship between the virulence of the organism as tested experimentally and the clinical course of the disease, nor can the critical termination of pneumonia be attributed to a sudden loss of virulence of the infecting pneumococcus.[2]

In the course of the bacteriological examination of the blood of patients described above, the virulence of the first cultures for white mice was regularly determined. No observations were made upon organisms obtained from the sputum because of the possible influence that the manipulations necessary for isolation may have upon the character of the organism. The custom has been to use the blood culture after twenty-four hours' growth, a period that has always permitted vigorous development. Varying dilutions of this culture with meat bouillon were made and injected intraperitoneally into white mice. The results are presented in table II.

Of the eighteen strains of pneumococcus isolated from the blood, thirteen were of extremely high original virulence for mice, doses of 0.00001 of a cubic centimeter or less of a twenty-four hour broth culture killing mice in less than thirty-six hours. Twelve of the individuals from whom highly virulent cultures were obtained died, and one recovered, convalescence being complicated by empyema. Three strains of medium virulence were obtained. One of these failed to kill mice in doses of 0.01 of a cubic centimeter, and two

[2] Luetscher, J. A., *Jour. Infect. Dis.*, 1911, ix, 287.

TABLE II.

Number of case.	Number of record.	Day of disease.	Recovery or death.	Virulence of culture.
I	423	5th	Died	0.01 c.c. D. 20 hrs. 0.001 c.c. D. 20 hrs. 0.00001 c.c. D. 24 hrs.
II	428	4th	Died	0.1 c.c. D. 40 hrs. 0.01 c.c. D. 3 dys. 0.0001 c.c. D. 3 dys. 0.000001 c.c. D. 6 dys.
III	438	2d	Died	0.01 c.c. D. 20 hrs. 0.0001 c.c. D. 20 hrs. 0.000001 c.c. D. 20 hrs.
IV	119	7th	Recovered	1.0 c.c. D. 36 hrs. 0.5 c.c. Survived.
V	453	Autopsy	Died	0.5 c.c. D. 18 hrs. 0.1 c.c. D. 18 hrs. 0.01 c.c. Survived. 0.0001 c.c. Survived.
VI	472	5th	Died	0.01 c.c. D. 20 hrs. 0.0001 c.c. Survived. 0.000001 c.c. D. 36 hrs.
VII	480	6th	Died	0.01 c.c. D. 20 hrs. 0.0001 c.c. D. 20 hrs. 0.000001 c.c. D. 22 hrs.
VIII	411	7th	Recovered	2.0 c.c. D. 14 hrs. 0.1 c.c. Survived. 0.01 c.c. Survived.
IX *P. mucosus*	484	7th	Died	0.01 c.c. D. 11 hrs. 0.0001 c.c. D. 17 hrs. 0.000001 c.c. D. 21 hrs.
X	487	3d	Died	0.0001 c.c. D. 20 hrs. 0.00001 c.c. D. 21 hrs. 0.000001 c.c. D. 24 hrs.
XI	525	4th	Died	0.0001 c.c. D. 18 hrs. 0.00001 c.c. D. 24 hrs. 0.000001 c.c. D. 24 hrs.
XII	533	6th	Died	0.0001 c.c. D. 24 hrs. 0.00001 c.c. D. 18 hrs. 0.000001 c.c. D. 24 hrs.
XIII	535	6th	Died	0.0001 c.c. D. 28 hrs. 0.00001 c.c. D. 28 hrs. 0.000001 c.c. D. 28 hrs.
XIV	551	2d	Recovered, empyema	0.0001 c.c. D. 18 hrs. 0.00001 c.c. D. 24 hrs. 0.000001 c.c. D. 26 hrs.

TABLE II (*Continued*).

Number of case.	Number of record.	Day of disease.	Recovery or death.	Virulence of culture.
XV *P. mucosus*	544	11th	Died	0.0001 c.c. D. 36 hrs. 0.00001 c.c. D. 36 hrs. 0.000001 c.c. D. 36 hrs.
XVI	447	7th	Died	0.0001 c.c. D. 22 hrs. 0.00001 c.c. D. 24 hrs. 0.000001 c.c. D. 24 hrs.
XVII	606	7th	Recovered	0.1 c.c. D. 36 hrs. 0.01 c.c. D. 3 dys. 0.0001 c.c. D. 4 dys. 0.00001 c.c. D. 5 dys. 0.000001 c.c. D. 3 dys.
XVIII	617	6th	Died	0.0001 c.c. D. 24 hrs. 0.00001 c.c. D. 24 hrs. 0.000001 c.c. D. 24 hrs.

killed animals in the higher dilutions only after considerable periods of time. Two patients, from whom these organisms were obtained, died, and one recovered without complications. Relatively avirulent strains were isolated from the blood in two cases, and in both instances the individuals recovered without complications.

The number of cases is entirely too few to permit any general deductions concerning the relation of the virulence of the pneumococcus to the course of the disease. As far as the indication goes, it would seem probable that individuals having an avirulent organism in the blood stand a better chance of recovery than those in whom the organism is of high virulence. That the latter quality necessarily indicates a fatal issue is disproven by case XIV, in which the individual recovered in spite of the presence of a considerable number of highly virulent pneumococci (sixty per cubic centimeter of blood). It is of some interest that most of the organisms isolated possessed a virulence comparable with that of the highly virulent laboratory strains. If the organisms obtained from the blood represent descendants of previous inhabitants of the mouth, there can be very little doubt that there has been a marked accession of virulence during the period of growth in the lungs and blood.

SUSCEPTIBILITY OF BLOOD AND SPUTUM STRAINS OF PNEUMOCOCCUS TO THE ACTION OF UNIVALENT ANTIPNEUMOCOCCUS SERUM.

Specific immune sera have been prepared by a number of investigators and have been used for the study of experimental pneumococcus infection in animals, and also in the treatment of lobar pneumonia and other pneumococcus infections in man. In animals it has been possible to prevent infection with pneumococcus when immune serum is given simultaneously with a lethal dose of pneumococci, and also to inhibit development of the organism when serum is given within three or four hours after infection. The effect of serum therapy upon lobar pneumonia in man has not been striking. Some observers have noticed a beneficial action and think they have noticeably affected the course of the disease. Study of the mortality statistics, however, of serum treated cases shows no appreciable difference in the death rate of individuals so treated and those untreated. With a better technique of serum administration, it may be possible to develop more striking effects, and with this end in view Neufeld[3] has urged the intravenous injection of large quantities of immune serum.

In this hospital, during the past year, an antipneumococcus serum of high potency has been developed by immunization of the horse with large doses of living virulent organisms. The serum was prepared by immunizing an animal against a single strain of pneumococcus, a typical organism of constant high virulence obtained from Professor Neufeld. The protective action of this univalent serum was tested against all strains of pneumococcus isolated from patients in the hospital. The organisms in most instances came from individuals suffering from lobar pneumonia. Pneumococci[4] have not as yet been divided into clearly defined biological groups, though it is known that a serum derived from a certain typical strain may fail to protect animals against infection with other strains that are morphologically and culturally characteristic. The following experiments were performed in order to determine the number of races of pneumococci against which a univalent serum would afford protection. White mice were used for experimental infection.

[3] Neufeld, F., and Händel, L., *Berl. klin. Wchnschr.*, 1912, xlix, 680.

[4] Neufeld, F., and Händel, *Ztschr. f. Immunitätsforsch., Orig.*, 1909, iii, 159.

TABLE III.

Number of organism.	Source.	Virulence.		Serum protection.		
		Amount of culture.	Result.	Amount of culture.	Amount of serum.	Result.
II	Lobar pneumonia blood	0.0001 c.c.	D. 24 hrs.	0.0001 c.c.	0.2 c.c.	D. 18 hrs.
		0.00001 c.c.	D. 24 hrs.	0.00001 c.c.	0.2 c.c.	D. 24 hrs.
		0.000001 c.c.	D. 24 hrs.	0.000001 c.c.	0.2 c.c.	Survived.
III	Broncho-pneumonia sputum	0.00001 c.c.	D. 24 hrs.	0.1 c.c.	0.2 c.c.	D. 24 hrs.
		0.000001 c.c.	D. 24 hrs.	0.01 c.c.	0.2 c.c.	D. 24 hrs.
				0.0001 c.c.	0.2 c.c.	Survived.
IV	Lobar pneumonia blood	0.0001 c.c.	D. 40 hrs.	0.1 c.c.	0.2 c.c.	Survived.
		0.00001 c.c.	D. 48 hrs.	0.01 c.c.	0.2 c.c.	Survived.
		0.000001 c.c.	D. 40 hrs.	0.001 c.c.	0.2 c.c.	Survived.
V	Lobar pneumonia blood	0.0001 c.c.	D. 18 hrs.	0.0001 c.c.	0.2 c.c.	D. 18 hrs.
		0.00001 c.c.	D. 18 hrs.	0.00001 c.c.	0.2 c.c.	D. 24 hrs.
		0.000001 c.c.	D. 24 hrs.	0.000001 c.c.	0.2 c.c.	D. 24 hrs.
VII	Lobar pneumonia sputum	0.0001 c.c.	D. 42 hrs.	0.1 c.c.	0.2 c.c.	Survived.
		0.00001 c.c.	D. 42 hrs.	0.01 c.c.	0.2 c.c.	Survived.
		0.000001 c.c.	D. 42 hrs.	0.001 c.c.	0.2 c.c.	Survived.
IX	Lobar pneumonia sputum	0.0001 c.c.	D. 30 hrs.	0.001 c.c.	0.2 c.c.	Survived.
		0.00001 c.c.	D. 46 hrs.	0.0001 c.c.	0.2 c.c.	Survived.
		0.000001 c.c.	D. 49 hrs.	0.00001 c.c.	0.2 c.c.	Survived.
X	Lobar pneumonia blood	0.0001 c.c.	D. 24 hrs.	0.1 c.c.	0.2 c.c.	Survived.
		0.00001 c.c.	D. 24 hrs.	0.01 c.c.	0.2 c.c.	Survived.
		0.000001 c.c.	D. 40 hrs.	0.001 c.c.	0.2 c.c.	Survived.
S. E. I	Endocar-ditis blood	0.001 c.c.	D. 22 hrs.	0.01 c.c.	0.2 c.c.	D. 18 hrs.
		0.00001 c.c.	D. 42 hrs.	0.001 c.c.	0.2 c.c.	D. 18 hrs.
		0.000001 c.c.	D. 22 hrs.	0.0001 c.c.	0.2 c.c.	D. 22 hrs.
XIV	Meningitis spinal fluid	0.3 c.c.	D. 20 hrs.	0.3 c.c.	0.2 c.c.	D. 20 hrs.
		0.1 c.c.	D. 24 hrs.	0.1 c.c.	0.2 c.c.	D. 20 hrs.
		0.01 c.c.	Survived.	0.01 c.c.	0.2 c.c.	Survived.
XV	Lobar pneumonia blood	0.0001 c.c.	D. 18 hrs.	0.0001 c.c.	0.2 c.c.	D. 18 hrs.
		0.00001 c.c.	D. 18 hrs.	0.00001 c.c.	0.2 c.c.	D. 18 hrs.
		0.000001 c.c.	D. 18 hrs.	0.000001 c.c.	0.2 c.c.	D. 18 hrs.
XVI	Lobar pneumonia blood	0.0001 c.c.	D. 5 dys.	0.001 c.c.	0.2 c.c.	D. 18 hrs.
		0.00001 c.c.	D. 24 hrs.	0.0001 c.c.	0.2 c.c.	D. 4 dys.
		0.000001 c.c.	D. 36 hrs.	0.00001 c.c.	0.2 c.c.	D. 5 dys.
				0.000001 c.c.	0.2 c.c.	Survived.
XVII	Lobar pneumonia sputum	0.0001 c.c.	D. 36 hrs.	0.0001 c.c.	0.2 c.c.	D. 36 hrs.
		0.00001 c.c.	D. 36 hrs.	0.00001 c.c.	0.2 c.c.	D. 36 hrs.
		0.000001 c.c.	D. 36 hrs.	0.000001 c.c.	0.2 c.c.	D. 36 hrs.
XIX *P. mucosus*	Lobar pneumonia blood	0.0001 c.c.	D. 18 hrs.	0.0001 c.c.	0.2 c.c.	D. 18 hrs.
		0.00001 c.c.	D. 26 hrs.	0.00001 c.c.	0.2 c.c.	D. 27 hrs.
		0.000001 c.c.	D. 36 hrs.	0.000001 c.c.	0.2 c.c.	D. 27 hrs.

TABLE III (*Continued*).

Number of organism.	Source.	Virulence.		Serum protection.		
		Amount of culture.	Result.	Amount of culture.	Amount of serum.	Result.
XX	Lobar pneumonia blood	0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	D. 20 hrs. D. 20 hrs. D. 20 hrs.	0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	0.2 c.c. 0.2 c.c. 0.2 c.c.	D. 20 hrs. D. 20 hrs. D. 20 hrs.
XXI	Lobar pneumonia sputum	0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	D. 24 hrs. D. 24 hrs. D. 36 hrs.	0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	0.2 c.c. 0.2 c.c. 0.2 c.c.	D. 3 dys. D. 3 dys. D. 3 dys.
XXII	Lobar pneumonia blood	0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	D. 18 hrs. D. 24 hrs. D. 24 hrs.	0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	0.2 c.c. 0.2 c.c. 0.2 c.c.	D. 18 hrs. D. 24 hrs. D. 18 hrs.
XXIII	Lobar pneumonia blood	0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	D. 24 hrs. D. 18 hrs. D. 24 hrs.	0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	0.2 c.c. 0.2 c.c. 0.2 c.c.	D. 18 hrs. D. 24 hrs. D. 24 hrs.
XXIV	Lobar pneumonia blood	0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	D. 28 hrs. D. 28 hrs. D. 28 hrs.	0.001 c.c. 0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	0.2 c.c. 0.2 c.c. 0.2 c.c. 0.2 c.c.	D. 18 hrs. Survived. Survived. Survived.
XXV	Lobar pneumonia blood	0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	D. 18 hrs. D. 24 hrs. D. 26 hrs.	0.1 c.c. 0.01 c.c. 0.001 c.c. 0.0001 c.c. 0.00001 c.c.	0.2 c.c. 0.2 c.c. 0.2 c.c. 0.2 c.c. 0.2 c.c.	D. 18 hrs. D. 3 dys. Survived. Survived. Survived.
XXVI *P. mucosus*	Lobar pneumonia lung	0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	D. 26 hrs. D. 24 hrs. D. 36 hrs.	0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	0.2 c.c. 0.2 c.c. 0.2 c.c.	D. 26 hrs. D. 36 hrs. D. 36 hrs.
XXVII	Lobar pneumonia blood	0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	D. 22 hrs. D. 24 hrs. D. 24 hrs.	0.1 c.c. 0.01 c.c. 0.001 c.c. 0.0001 c.c.	0.2 c.c. 0.2 c.c. 0.2 c.c. 0.2 c.c.	D. 18 hrs. Survived. Survived. Survived.
XXVIII	Lobar pneumonia blood	0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	D. 4 dys. D. 5 dys. D. 3 dys.	0.1 c.c. 0.01 c.c. 0.001 c.c. 0.0001 c.c.	0.2 c.c. 0.2 c.c. 0.2 c.c. 0.2 c.c.	D. 3 dys. Survived. Survived. Survived.
XXIX	Lobar pneumonia blood	0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	D. 24 hrs. D. 24 hrs. D. 24 hrs.	0.0001 c.c. 0.00001 c.c. 0.000001 c.c.	0.2 c.c. 0.2 c.c. 0.2 c.c.	D. 18 hrs. D. 24 hrs. D. 24 hrs.

Wherever the original virulence of the pneumococcus was low, it was raised by successive animal passages until a high degree of virulence was reached. Twenty-four hour broth cultures made from the heart's blood of passage animals were used for infection. The protective dose of serum and given quantities of diluted culture

were injected simultaneously into the peritoneal cavity. Protection was deemed complete when animals survived inoculation longer than five days (table III).

A univalent serum developed by immunization against pneumococcus N–I was tested for protection against twenty-three strains of pneumococcus. Of these organisms two belonged to the group that is commonly designated as *Pneumococcus* or *Streptococcus mucosus;* one was an organism isolated from the spinal fluid that had certain characteristics belonging to the pneumococcus, and other features that suggested a relationship to streptococcus; and another organism was one obtained from the blood in a case of infectious endocarditis. The latter on isolation resembled streptococcus, but after several passages through animals, acquired many of the characters of pneumococcus. The remaining nineteen strains were all typical pneumococci obtained either from the blood or sputum of patients with pneumonia. Out of fourteen strains of unquestionable pneumococci obtained by blood culture, the univalent serum gave some degree of protection against eight, about 57 per cent. Of five sputum strains, four were susceptible to the protective action of the serum. Of the total number of twenty-three strains against which the serum was tested, including two strains of *Pneumococcus mucosus* and two organisms of doubtful identity, it was proved that protective antibodies were present in twelve instances, slightly more than 50 per cent. The amount of protection afforded by the serum against these strains varied from a grade practically equal to that found when pneumococcus N–I was used for infection to protection against a single lethal dose, or in one instance to simple prolongation of the period of life of the infected animals. In eight instances the serum protected against 1,000 or more lethal doses, and in four the protection was of a lower grade. An antipneumococcus serum, derived by immunization of animals with a certain single type strain of pneumococcus, therefore protects against a considerable proportion of the races ordinarily met with in lobar pneumonia.

If such a univalent serum is used therapeutically in human pneumonia, one knows beforehand that one half or more of the individuals treated must derive no benefit from its use, because of the probable insusceptibility of many of the infecting organisms to the pro-

tective action of the serum. It is not unlikely that if a multiplicity of strains are used indiscriminately in the preparation of an immune serum, the degree of immunity to any single strain would be proportionately low. In view of this supposition, further studies of the biological relationship of different strains of pneumococcus are being made in order to determine the widest range of protection that can be obtained by the use of a minimal number of distinct races. Should it be found that a reasonably small number of strains would yield protective bodies for a large percentage of the varieties of pneumococcus ordinarily met with, it might be possible to immunize single animals to individual strains and in this way obtain sera of the highest protective value for suitable cases. In human beings a fairly rapid test of the susceptibility of the organism concerned could be made, and in this way a serum could be selected for treatment which would give the greatest probability of success.

SUMMARY.

Studies of the bacteriology of the blood were made in thirty-seven cases of lobar pneumonia. The pneumococcus was isolated from the blood in approximately 50 per cent. of the cases studied. The course of infection in individuals with pneumococcus in the blood was more severe than in those in which no organism could be cultivated from the blood. 77 per cent. of the patients with positive blood cultures died, and 79 per cent. of patients with negative blood cultures recovered. In fatal instances of pneumonia, where the pneumococcus was found in the blood, the number of organisms per cubic centimeter of blood was very high in the last stage of the disease. In individuals dying of pneumonia without blood infection, the disease was characterized by a rapid spread of the local process in the lungs. It is not unlikely that the symptoms of collapse, developing on the fifth or sixth day of lobar pneumonia, are often the expression of serious invasion of the blood by the pneumococcus. In other instances, they mark an extension of the local process in the lungs.

Strains of pneumococcus isolated from the blood of patients with lobar pneumonia were usually of high animal virulence. In a few instances where the organism isolated from the blood was of low virulence for animals, the patients recovered.

The protective power of a univalent antipneumococcus serum was tested against nineteen strains of typical pneumococcus and against four strains of closely allied organisms. The serum manifested some degree of protection against twelve out of nineteen strains of typical pneumococci. No protection was observed against the atypical organisms. In eight instances the degree of protection obtained was high, in three low, and in one there occurred only a prolongation of the period of life of the inoculated animal.

COAGULATION TIME OF THE BLOOD IN LOBAR PNEUMONIA.*

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Observation of the variation in time within which the blood coagulates when removed from its normal surroundings is of importance in the study of certain diseases, and accurate determination of the factors involved in producing any variation in the normal coagulation time of the blood would, no doubt, throw some light upon the nature of the pathological processes concerned. A large number of methods designed to estimate the time of coagulation under normal and pathological conditions have been devised. Many of these methods give fairly accurate results in the hands of their originators, but the time of normal coagulation differs considerably when determined by different methods, so that comparison of one observer's results with those of another is unsatisfactory.

Morowitz[1] has devoted much study to the relation of different elements and to the sequence of events in the process of blood coagulation. The most satisfactory and adequate explanation of the phenomenon, however, has recently been offered by Howell,[2] who has carefully traced the various stages in the reaction, which finally results in the conversion of fibrinogen into fibrin. All the elements necessary for coagulation are present in the circulating blood. The inception of the process is prevented, however, by the presence of a substance, antithrombin, which binds the prothrombin, thus preventing its activation within the vessels by the calcium salts present. When shed blood is exposed to tissue juices or foreign surfaces, a body designated thromboplastin, originating either from tissue juices or the disintegration of certain cellular elements in the blood, develops. Thromboplastin, by its ability to combine with antithrombin, frees the previously bound prothrombin. This immediately reacts with the calcium salts of the blood with the liberation of active thrombin, which then precipitates the fibrinogen as fibrin. This explanation emphasizes the probability that failure of the coagulative power, or changes in the coagulation time of the

* Received for publication, August 1, 1912.

[1] Morowitz, P., Handbuch der biochemischen Arbeitsmethoden, 1911, v, 223.

[2] Howell, W. H., *Am. Jour. Physiol.*, 1911–12, xxix, 187.

blood, are dependent upon variation in some one or more of the factors described. Quantitative estimation of the substances concerned is possible and would yield more valuable results than simple determination of the coagulation time, even were the methods for the latter observation uniform. Whipple[3] has recently applied such a procedure to the study of certain diseases and urges a classification of conditions associated with hemorrhage upon the basis of the specific element responsible for the change in coagulative power.

In the present paper are given the results obtained from a study of the coagulation time of the blood in a number of cases of lobar pneumonia. On account of the large amount of time required, quantitative estimations of the thrombin, antithrombin, and fibrinogen were not made, so that it is possible to explain only by inference the prolongation of the coagulation time observed during the acute stage of pneumonia.

METHOD.

The method used for determination of the coagulation time was a comparatively simple one. An attempt was made to guard against certain obvious sources of error. A fairly large quantity of blood, about seven cubic centimeters, was used for each determination in order that the proportion of volume to the surface exposed to contact with foreign substances might be as great as possible.

The blood was obtained by rapidly filling an all glass syringe, having a needle of large calibre, with blood directly from the median basilic vein of the patient. A stoppered glass tube was then filled from the syringe to within about two centimeters of the top. The tube had previously been inserted through a rubber stopper into a large glass cylinder containing water kept at 38° C. The average time for the complete operation was about forty-five seconds. The apparatus was then carried to a room kept constantly at 38° C., and after the lapse of some minutes the tube was carefully rotated every thirty seconds. The time of coagulation was measured from the period at which half the quantity of blood had been withdrawn from the vein to the point at which the blood failed upon rotation to flow from one end of the tube to the other. Great care was taken to prevent the presence of air bubbles upon the upper surface of the blood, as these accelerate the formation of a clot at this point. All glassware with which the blood came in contact was maintained as nearly as possible at body temperature and was kept scrupulously clean. The specimen of blood was taken by venous puncture in order to prevent contact with lacerated tissue.

It had been assumed, that if prolongation of the coagulation time occurred, such a condition would probably be dependent upon an

[3] Whipple, G. H., *Arch. Int. Med.*, 1912, ix, 365.

excess of antithrombin. If such were the case, the ordinary method of obtaining drops of blood from a skin puncture would expose it to contamination with thromboplastin, and inasmuch as the excess of antithrombin might be very slight, a sufficient quantity of thromboplastic substances might be absorbed during the formation of a small drop to mask entirely the presence of a slight excess of antithrombin. It is a well known fact that successive drops from the same skin puncture clot with increasing rapidity. This can hardly be looked upon as comparable to the shortening of coagulation time which occurs during hemorrhage. The blood while in the vessels shows no tendency to clot, and the time within which it coagulates when removed depends upon the variety and nature of external conditions, so that different methods in the main give variable results. No special advantages are claimed for the method used in this study. It is possible for the same individual to obtain comparable results if variations in technique are carefully guarded against.

The idea has become current that the coagulation time of the blood is shortened during the acute stage of lobar pneumonia. This opinion has developed in spite of the observations of Hayem,[4] Pye-Smith,[5] and Coleman,[6] that the clotting time is prolonged in pneumonia. Cohen,[7] in a study of six pneumonia patients, found the average time slightly shortened. The formation of white clots in the heart and vessels of patients dying of lobar pneumonia is a common observation at autopsy. In this hospital a marked sedimentation of the red cells, with the formation of a buff coat, occurred when tubes of blood obtained from pneumonia patients were allowed to stand. Such observations are infrequent in the case of normal blood and suggest that the coagulation time of the blood in pneumonia is sufficiently delayed to allow a settling of the heavier cellular elements.

In the course of some recent experiments in which rabbits were infected with massive doses of pneumococcus, the fact was noted that when the animals died, after the lapse of only a few hours, the blood had already become practically incoagulable. These ob-

[4] Hayem, G., Du sang et de ses altérations anatomiques, Paris, 1889, 323.
[5] Pye-Smith, P. H., Allbutt and Rolleston, System of Medicine, 1898, v, 91.
[6] Coleman, C. J., *Biochem. Jour.*, 1907, ii, 184.
[7] Cohen, M. S., *Arch. Int. Med.*, 1911, viii, 684, 820.

servations seemed to indicate the probability of a prolongation of coagulation time during lobar pneumonia. The following cases demonstrate in most instances a definite increase in the clotting time of the blood during the acute stage of lobar pneumonia with a gradual return to normal during the period of convalescence.

Case 1.—411. Severe infection. Recovered. Lysis on the ninth day of disease.

Dec. 16, 1911, three days before lysis.	Dec. 19, 1911, during lysis.	Jan. 3, 1912, temperature normal.
Coagulation time:		
10 min.	11 min., 10 sec.	6 min., 30 sec.

Case 2.—418. Mild infection. Recovered. Lysis on the fourth day of disease.

Dec. 24, 1911, two days before lysis.	Dec. 29, 1911, one day after lysis.	Jan. 8, 1912, temperature normal.
Coagulation time:		
16 min.	10 min.	7 min.

Case 3.—409. Severe infection. Died on the fourth day of disease.

Jan. 4, 1912, second day of disease.
Coagulation time:
17 min., 10 sec.

Case 4.—413. Severe infection. Died on the fourth day of disease.

Jan. 7, 1912, third day of disease.
Coagulation time:
13 min.

Case 5.—423. Severe infection. Died on the seventh day of disease.

Jan. 14, 1912, fifth day of disease.	Jan. 16, 1912, two hours before death.
Coagulation time:	
15 min.	18 min.

Case 7.—454. Mild infection. Recovered. Crisis on the third day of disease.

Jan. 21, 1912, day of crisis.	Jan. 28, 1912, seven days after crisis.
Coagulation time:	
12 min.	10 min., 30 sec.

Case 8.—439. Severe infection. Died on the tenth day of disease

Jan. 22, 1912, sixth day of disease.	Jan. 25, 1912, ninth day of disease.
Coagulation time:	
11 min.	12 min.

Case 9.—438. Severe infection. Died on the fourth day of disease.

Jan. 24, 1912, second day of disease.
Coagulation time:
9 min.

Case 10.—503. Moderately severe infection. Crisis on the seventh day of disease.

Jan. 25, 1912, four days before crisis.	Jan. 29, 1912, one day before crisis.	Jan. 30, 1912, day of crisis.	Feb. 14, 1912, two weeks after crisis.
Coagulation time:			
11 min., 45 sec.	12 min.	9 min.	9 min.

Case 11.—460. Severe infection. Died on the fifth day of disease.

Feb. 2, 1912, third day of disease.	Feb. 4, 1912, fifth day of disease.
Coagulation time:	
8 min., 45 sec.	11 min., 30 sec.

Case 12.—472. Severe infection. Died on the fifth day of disease.

Feb. 12, 1912, third day of disease.	Feb. 14, 1912, fifth day of disease.
Coagulation time:	
13 min., 30 sec.	12 min., 45 sec.

Case 13.—497. Moderately severe infection. Recovered. Crisis on the seventh day of disease.

Feb. 13, 1912, two days before crisis.	Feb. 15, 1912, day of crisis.	Feb. 18, 1912, three days after crisis.
Coagulation time:		
14 min., 15 sec.	11 min., 30 sec.	8 min., 30 sec.

Case 14.—484. Severe infection. Died on the eighth day of disease.

Feb. 19, 1912, third day of disease.	Feb. 23, 1912, seventh day of disease.
Coagulation time:	
9 min., 15 sec.	13 min., 30 sec.

Case 15.—502. Severe infection. Recovered. Crisis on the eighth day of disease.

Feb. 19, 1912, seven days before crisis.	Feb. 21, 1912, five days before crisis.	Feb. 27, 1912, day of crisis.	March 11, 1912, thirteen days after crisis.
Coagulation time:			
11 min.	9 min., 15 sec.	7 min., 30 sec.	7 min., 30 sec.

Case 16.—501. Moderately severe infection. Recovered. Crisis on sixth day of disease.

Feb. 19, 1912, four days before crisis.	Feb. 21, 1912, two days before crisis.	Feb. 23, 1912, one day after crisis.	March 11, 1912, seventeen days after crisis.
Coagulation time:			
8 min., 15 sec.	10 min., 15 sec.	11 min.	6 min., 45 sec.

Case 17.—480. Severe infection. Died on the seventh day of disease.

Feb. 21, 1912, sixth day of disease.
Coagulation time: 13 min., 45 sec.

Case 18.—553. Moderately severe infection. Recovered. Pseudocrisis on fifth day of disease. Crisis on eighth day of disease.

March 5, 1912, five days before crisis.	March 7, 1912, day of pseudocrisis.	March, 11, 1912, one day after crisis.	March 26, 1912, sixteen days after crisis.
Coagulation time.			
11 min., 30 sec.	9 min., 30 sec.	11 min., 45 sec.	8 min.

Case 19.—405. Mild infection. Recovered. Crisis on eighth day of disease.

Dec. 14, 1911, one day before crisis.	Dec. 15, 1911, one day after crisis.	Dec. 21, 1911, seven days after crisis.	Jan. 2, 1912, three weeks after crisis.
Coagulation time:			
6 min., 15 sec.	6 min., 40 sec.	7 min.	8 min., 30 sec.

Case 20.—401. Severe infection followed by empyema.

Dec. 21, 1911, fourth day of disease.
Coagulation time: 12 min., 30 sec.

In seven instances the coagulation time of the blood in approximately normal individuals was determined by the method described. The specimens of blood were obtained from treated cases of syphilis in whom there was no evidence of syphilitic disease of the liver. The average coagulation time of the blood of these individuals was seven minutes and thirty-nine seconds. The shortest time observed was five minutes and fifteen seconds, and the longest time nine minutes. The average coagulation time of patients with pneumonia during the acute stage of the disease was twelve minutes and twenty-seven seconds. The shortest time was six minutes and fifteen seconds, and the longest time eighteen minutes. The average coagulation time at the time of discharge in patients who recovered was seven minutes and thirty-two seconds. The shortest time observed was six minutes and thirty seconds, and the longest, excepting one patient whose blood had last been tested some days before discharge, was nine minutes.

From these examples it is seen that the coagulation time of the blood is definitely prolonged during the acute stage of pneumonia. In one instance, case 20, a very mild infection involving part of one

lobe, the coagulation time during the height of the disease was possibly slightly less than normally. The degree of prolongation of the coagulation time seems to have no definite relationship to the severity of the pneumonic process. The coagulation time tends to become shorter at about the time of the crisis, but probably does not return to normal for some time afterward. At the time of discharge all patients show a coagulation time that is within normal limits, and the average coagulation time at this period corresponds very closely to that observed in approximately normal individuals.

The factor which causes the lengthening of coagulation time in pneumonia was not decisively determined because quantitative estimations of the various elements entering into the coagulation of the blood could not be made. For the following reasons, however, it was believed probable that the long coagulation time was caused by an increase in the antithrombin of the blood. In rough estimations made by centrifugalization, the proportion of the solid element of the clot to the serum has been found to be markedly increased during lobar pneumonia. This striking increase, of course, cannot be accounted for by an increase in the cellular elements and must be due to an increased amount of fibrinogen in the circulating blood. That an increase in the amount of fibrinogen occurs during pneumonia is known and has received experimental confirmation in pneumococcus infections of animals. It is not unlikely that the characteristically large fibrin content of pneumonic exudates depends upon the increased fibrinogen of the blood. Dr. Peabody, in this hospital, has shown that the decrease in calcium content is very slight and is inadequate to explain the changes in coagulation time. Howell[8] has determined that the formation of fibrin from fibrinogen is not a fermentative process but is a quantitative reaction between thrombin and fibrinogen. Inasmuch as a large firm clot always forms in pneumonic blood, it would seem unlikely that there is a deficiency of thrombin, but that the delay is caused by the presence of some inhibitory substance such as antithrombin. In addition, Whipple[9] has recently demonstrated by accurate methods, that in an analogous infection the delay in coagulation time is occasioned by an increase in antithrombin.

[8] Howell, W. H., *Am. Jour. Physiol.*, 1910, xxvi, 453.

[9] Whipple, G. H., *loc. cit.*

The liver is generally accepted as the principal source of the antithrombin of the blood. Meek[10] has recently confirmed the results of other observers in showing that the liver is most important in the regeneration of circulating fibrinogen. The increase in both of these elements of the blood during lobar pneumonia would seem to be due to a common stimulus affecting principally the liver. It is well known that injection into the circulation of animals of certain degradation products of protein induces a prolongation of the coagulation time of the blood. In lobar pneumonia opportunity is afforded for absorption from the lung of large quantities of such substances. Inasmuch as it is shown in the present paper that at the time of the crisis, when probably the greatest quantity of these substances reach the circulation, the coagulation time tends to decrease, it is unlikely that they are the chief factors concerned in the lengthening of coagulation time. The result is more probably produced by some specific effect upon the liver of intoxicating substances originating from the infecting organism.

SUMMARY.

The coagulation time of the blood is generally prolonged during the acute stage of lobar pneumonia, returning to normal during the period of convalescence.

There seems to be a simultaneous increase in the quantity of circulating fibrinogen. The lengthening of the coagulation time is probably due to an increased formation of antithrombin. The source of the increased antithrombin and fibrinogen is probably the liver, and the stimulus to increased production of these two substances is due to the nature of the infecting organism.

[10] Meek, W. J., *Am. Jour. Physiol.*, 1912, xxx, 161.

THE CARBON DIOXIDE CONTENT OF THE BLOOD IN PNEUMONIA.*

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As a result of experimental study and of a limited amount of clinical investigation, it has long been known that fever, or more accurately infection, is very generally associated with a diminution in the carbon dioxide content of the blood. This low carbon dioxide content has been accepted, probably correctly, as an index of acidosis. Apparently the values for carbon dioxide in the blood in fever are not infrequently within the range which is found in the more typical acidosis of diabetes. The object of the present study was to determine more accurately than has yet been done the constancy and extent of the variations in the carbon dioxide content of the blood in pneumonia, the possible relation of such variations to the course or severity of the disease, and more especially the relation of the carbon dioxide content to certain changes in the urine in this disease. Analysis of arterial blood, would, of course, be of much more value than of venous blood, but it is quite impossible to obtain samples of arterial blood from patients except under unusual circumstances. It will be noted, however, that the experimental studies on arterial blood agree well with the clinical studies on venous blood. On account of the recognized bearing of the carbon dioxide content of the blood on acidosis, the nitrogen and ammonia of the urine were quantitated. Determinations of "alkali retention" (Henderson and Adler) were also made, as this method is stated to be an index of acidosis. The relation between chlorine retention and carbon dioxide was studied, and observations were also made on the "total acidity" (Folin), phosphorus, and acetone in the urine.

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The first observations on the gases of the blood in fever were made by Pflüger in 1868 in the course of a series of experiments on dogs. Small operations were performed and the wounds healed with pus formation. Analysis of the blood showed a diminution in the contents of both oxygen and carbon dioxide. Senator[1] made determinations of the gases in the blood of a dog injected with pus. Four hours after injection the temperature had risen to 38.7° C., and the arterial blood showed a fall of 5.6 per cent. in the content of carbon dioxide. The oxygen had remained normal. Reynard's[2] experiments gave variable results. In one instance fever was produced by the injection of putrifying urine, and a fall in the oxygen and rise in carbon dioxide content of the arterial blood were noted. In two experiments the oxygen was increased and the carbon dioxide diminished. The latter he considered due to the accelerated respiration. In a dog in which septicemia was produced by injecting putrified blood into the femoral vein a fall of both oxygen and carbon dioxide occurred. Geppert[3] made a more extensive study of the arterial blood in fever in dogs. The oxygen content he found not to vary from the normal. The carbon dioxide content, on the other hand, dropped considerably, and the diminution appeared to run approximately proportional to the height of the fever. The fall in carbon dioxide even after the onset of a very intense fever was somewhat delayed. Geppert explained the results by supposing that in fever there is a diminution in the alkalinity of the tissues and a subsequent corresponding change in the composition of the blood. He cites as an analogous condition the low carbon dioxide content, associated with a rise of temperature, caused by severe muscular exertion. "As a result of prolonged contractions, the muscle becomes acid, allows acid products to get into the blood, diminishes its alkalinity, and thus explains the fall in carbon dioxide."

Minkowski[4] made extensive contributions to the subject. Geppert's results had been criticized on the ground that the low carbon dioxide was dependent on rapid respiration. Hence Minkowski first confirmed his observations as to the constancy of the low carbon dioxide in fever, and then showed that the change is quite independent of respiratory rate. Minkowski suggested, as did Geppert, that the essential factor is a change in the composition of the blood,—a diminution of the substances which bind carbon dioxide. Indirect evidence of acid formation in fever is given by the high ammonia output, the frequency with which acetone is found in the urine, and the finding by Minkowski of lactic acid in small amounts in the blood of febrile dogs. Minkowski repeated the experiments of Walter[5] who found a diminution in the carbon dioxide of the blood of animals fed on mineral acids. Rabbits, which on account of their herbivorous diet have little ammonia at their disposal with which to neutralize acids, showed a greater fall of carbon dioxide than did dogs which were fed on a meat diet. In fever the fall in the carbon dioxide content of the blood is greater in rabbits

[1] Senator, H., Untersuchungen über den fieberhaften Process und seine Behandlung, Berlin, 1873.

[2] Reynard, Recherches expérimentales sur les variations pathologiques des combustions respiratoires, Paris, 1879.

[3] Geppert, J., *Ztschr. f. klin. Med.*, 1880–1, ii, 355.

[4] Minkowski, O., *Arch. f. exper. Path. u. Pharmakol.*, 1885, xix, 209.

[5] Walter, F., *Arch. f. exper. Path. u. Pharmakol.*, 1877, vii, 148.

than in dogs. The organism in fever is consuming its own tissues and is thus essentially on a meat diet,—a diet associated with a high ammonia output in which the alkali present is not sufficient to cover the overproduction of acids formed. Another factor suggested for the withdrawal of available alkali for transporting carbon dioxide is the passage of alkali from the serum into the tissue cells, where it is bound by the acid products of decomposition. The retention of sodium in fever was cited. Finally Minkowski found a similar low value for the carbon dioxide in the rise of temperature produced artificially in overheated animals. While probably in part due to increased respiratory rate, other factors, notably metabolic changes and muscular rigor, play an important part, in which again the essential feature is a diminution in the alkalinity of the blood.

That the carbon dioxide content of the blood is diminished during fever had been definitely established by animal experimentation when Kraus[6] made the first observations on man. His studies embraced a series of fourteen patients with fever of various types. Typhoid and tuberculosis composed the long fevers, and erysipelas, scarlet fever, and pneumonia the short ones. The blood was obtained from the median vein. In general the results were a confirmation of those obtained in animals. While the normal percentage of carbon dioxide in the blood varies, according to him, from 26 to 40.3 per cent. by volume, most of the normal figures run between 31.34 and 35.96 per cent. During fever, however, the carbon dioxide content was between 9.84 and 20.34 per cent. An exception to this was formed by two cases of pneumonia. One of these, on the fifth day of the disease, had a carbon dioxide content of 22.16 per cent., and the other, on the sixth day, a content of 29.2 per cent.,—thus within normal limits. Kraus suggests that the relatively high carbon dioxide content of the blood in the cases of pneumonia may be due to the fact that the sudden lessening of available respiratory surface by the formation of the pneumonic exudate interferes with the excretion of carbon dioxide in the lungs. He found no especial relation between the height of temperature and the diminution of carbon dioxide and suggested that temperature is in general no index of the severity of an infection, but possibly the low carbon dioxide, indicating the more essential metabolic changes in fever, might be some measure of the severity of the disease. In the majority of his cases one analysis of the blood was made but in one instance of pneumonia a second analysis was made a few hours after the crisis, and in three cases of typhoid fever analyses were made at a time when the temperature had been brought down to normal by administering antipyretics. As in none of these was there any marked rise in the carbon dioxide, he concluded that the fall of the fever is not immediately followed by a return of the carbon dioxide to normal. In two cases of erysipelas, however, the second analysis was made after one day without fever, and after the patients had begun to take nourishment. In both the carbon dioxide had risen to normal. Kraus agreed with the former investigators that the diminished carbon dioxide content of the blood in fever is the expression of a lowered alkalinity of the blood. This view, he believed, received support from his method of direct titration of the blood.

[6] Kraus, F., *Ztschr. f. Heilk.*, 1889, x, 106.

METHODS.

For the purposes of a study in which it was intended that frequent analyses of the blood of patients should be made, and in which very small variations in the results would be of little or no importance, it was desirable to have a method which allowed accurate blood-gas determinations in small amounts of blood, and which was simple enough to be applicable for clinical use. The method described by Barcroft and Haldane[7] fulfills these requirements. The apparatus and the method of using it have been described so well by its originators that nothing need be added on this point. In general it is as follows: About 1 c.c. of blood, taken under precautions to exclude contact with air, and prevented from coagulating, is put into a small glass bottle which connects with a water manometer. The blood is laked by ammonia solution. By a special device inside the bottle, potassium ferricyanide is added to the laked blood and the oxygen set free. The increase of pressure caused by the gas is measured by the manometer. After resetting the manometer, tartaric acid is added to the blood mixture and the carbon dioxide set free. From the change of pressure shown by the manometer and from the previously determined volume of the bottle and manometer, the volume of gas liberated is calculated. Corrections are made for the solutions used, for temperature and barometric pressure, and for the absorption of carbon dioxide by the fluid mixture in the bottle. The results are then reported in volumes of gas reduced to a pressure of 760 mm. and a temperature of 0° C.

In this work some of the modifications of the original apparatus as devised by Brodie[8] were used. The "modified blood-gas apparatus" is easier to work with than the original form, and the substitution of a solution of bile salts for water in the manometer greatly simplifies the problem of keeping the manometer absolutely clean. Brodie's method for collecting, measuring, and delivering the blood was tried, but proved rather too complicated for use in connection with patients. Glass syringes provided with metal cases, of the form described by Barcroft and Haldane, were therefore used. In these syringes the blood is mixed with a known amount of 2 per cent. potassium oxalate solution and an accurately measured quantity of blood and oxalate delivered into the gas apparatus. In nearly every instance two separate samples of blood were taken. One disadvantage of this method is that it involves puncturing the skin twice, but with sharp needles the discomfort is slight. Another possible disadvantage is suggested by the experiments of Pflüger,[9] who obtained somewhat varying results in his sets of blood-gas analyses, unless the samples of blood were taken simultaneously. He states that the difference might be as great if the second sample was taken immediately after the first as if the two samples were taken some hours apart. The blood was taken from one of the veins at the bend of the elbow. On account of the effect of stasis on the blood-gases, it was a general rule that no compression of the arm or obstruction of the circulation should be caused. In the rare cases in which it was not possible to enter the vein

[7] Barcroft, J., and Haldane, J. S., *Jour. Physiol.*, 1902, xxviii, 232; Barcroft, J., and Morawitz, P., *Deutsch. Arch. f. klin. Med.*, 1908, xciii, 223.

[8] Brodie, T. G., *Jour. Physiol.*, 1909–10, xxxix, 391.

[9] Pflüger, E., *Arch. f. Anat. u. Physiol.*, 1868, i, 61.

without some distension of it, compression was made for a very short time, and released as soon as the needle was in the vein. The blood was then withdrawn slowly and only after enough time had elapsed for the circulation to readjust itself.

The application of this method of blood-gas analysis to clinical work presents some difficulties at first, but the results obtained are, for the most part, very satisfactory. The two controls usually checked accurately and almost always gave results within the necessary limits of accuracy of the experiment. Of sixty-five consecutive pairs of analyses of the carbon dioxide in the blood, twenty-four agreed within less than 1 per cent. by volume, forty-three within 2 per cent., and fifty-one within 3 per cent.

According to Loewy,[10] the arterial blood of man contains between 39 and 43 per cent. of carbon dioxide at a tension of 30 to 40 mm. of mercury, while venous blood contains from 43 to 50 per cent. of carbon dioxide at a tension of 40 to 50 mm. of mercury. The figures obtained in this investigation for the normal carbon dioxide content of the venous blood are somewhat higher than those given by Loewy, but they are very constant, both in healthy individuals and in the patients after their metabolic processes had returned to normal. In general, the normal carbon dioxide of the venous blood varied between 54 and 58 per cent.

The methods used in the analysis of the urine were as follows: "Alkali retention" was determined according to the method of Henderson and Adler.[11] A definite quantity of urine, under standard conditions, is titrated with one-tenth normal sodium hydrate solution, using neutral red as an indicator, until it is of the same reaction as a standard solution which is made up to the reaction of blood. This titration is supposed to give an index of the amount of acid excreted by the kidney, and corresponds to the amount of base saved to the body. To this figure is added the ammonia excretion, expressed also in terms of one tenth normal solution. The sum of the two measures "the effective work of the kidney in saving basic substances for the further neutralization of acid and transport of carbonic acid." The other determinations were done by the usual methods,—total nitrogen by Kjeldahl's, ammonia by Folin's,[12] chlorine by the Volhard method,[13] phosphorus by titration with uranium nitrate solution,[14] acetone by Lieben's test, and "total acidity" by the method of Folin.[15]

THE CARBON DIOXIDE CONTENT OF THE VENOUS BLOOD.

Observations on the carbon dioxide content of the venous blood were made on twenty-six cases of pneumonia. Of these, eleven

[10] Loewy, A., in von Koranyi, A., and Richter, P. F., Physikalische Chemie und Medizin, Leipzig, 1907, i, 255.

[11] Henderson and Adler, *Jour. Biol. Chem.*, 1909, vi, p. xxxviii; Adler, H. M., and Blake, G., *Arch. Int. Med.*, 1911, vii, 479.

[12] Folin, O., *Am. Jour. Physiol.*, 1905, xiii, 45.

[13] Harvey, S. C., *Arch. Int. Med.*, 1910, vi, 12.

[14] Neubauer-Huppert, Analyse des Harns, 11th edition, Wiesbaden, 1910, i, 138.

[15] Folin, O., *loc. cit.*

were fatal cases. In all, ninety-one analyses were made. With the exception of two cases, which will be discussed later, the carbon dioxide was found to be regularly diminished during the febrile period. In most cases the carbon dioxide content ran from 40 to 50 per cent. in the acute stage of the disease, but not infrequently it was from 50 to 53 per cent., or only very slightly below normal. The lowest observation made was 29.01 per cent.

No definite relationship could be made out between the carbon dioxide content of the blood and either temperature, pulse, or respiration, except that the lowest values for carbon dioxide were apt to be found associated with very rapid respiration. Nor was there any clear-cut relation between the carbon dioxide and the extent of lung involved, or the severity of the disease. Four cases, however, in which observations were made within two hours of death, are of considerable interest, as they show that the tendency is for the carbon dioxide content to be very low in the terminal stage of the disease. When, however, circulatory disturbances or local processes in the lungs interfere with the respiratory exchange of gases, the carbon dioxide may be relatively higher. In case XIX the chest was free from râles two hours before death, and the oxygen content of the blood was normal. Gaseous exchange in the lungs was thus apparently unaffected. The carbon dioxide content was 29.01 per cent., the lowest value found in the whole series. Case XVI was very cyanotic and had a general edema of the lungs one hour before death. An exceedingly low oxygen content of the venous blood suggests that oxygen was being imperfectly taken up in the pulmonary capillaries, and makes it probable that the excretion of carbon dioxide was similarly interfered with. In spite of this, the carbon dioxide was only 35.53 per cent. The same conditions, pulmonary edema and low oxygen content, prevailed in case XII two hours before death, and here again the carbon dioxide value was one which would be considered low, even if there were no hindrance to the excretion of carbon dioxide in the lungs. Similarly, case XXIV had, on clinical examination thirty minutes before death, marked cyanosis and many coarse tracheal râles. At this time the oxygen content of the venous blood was only 2.07 per cent., and the carbon dioxide content was

40.46 per cent. Thus the three last cases show a carbon dioxide content which is absolutely low and may be considered to be relatively very low. Beyond this, there is little association between the severity of the disease and the carbon dioxide of the blood, except that the mild cases with a short course usually showed the least marked deviations from the normal.

The relation of the carbon dioxide content of the blood to the course of the disease is somewhat variable. Unfortunately, no observations were made on the first or second days. Several cases, however, notably Nos. III, VIII, IX, and XXI, show that the carbon dioxide is, in general, nearer normal in the first days of the disease, and then gradually sinks, so that the minimum is at about the height of the disease or at the time of the crisis. After the crisis the carbon dioxide usually begins to show a rise towards normal, but occasionally very low, and even the lowest figures are found some days after the patient has become afebrile. Thus case XXI had a carbon dioxide content of 43.08 per cent. four days after the crisis, and case III one of 45.23 per cent. on the fifth day of normal temperature. Both of these patients had been taking a good amount of nourishment for several days, so that the low carbon dioxide can scarcely be referred to a starvation acidosis. These cases bring out the fact that the low carbon dioxide content of the blood may persist for a considerably longer period after the fall of temperature than was noticed by Kraus, and they show definitely that the changes in the carbon dioxide bear no direct relation to temperature itself, but are rather in line with the other metabolic changes which are associated with infection and which are sometimes more marked after the fall of temperature than during the febrile period itself. Chief among the evidences of these changes, as shown in the urine, are the high nitrogen and ammonia excretion ("epicritical excretion") and the low chloride excretion.

THE RELATION OF THE CARBON DIOXIDE CONTENT OF THE BLOOD TO THE EXCRETION OF AMMONIA AND CHLORINE IN THE URINE.

The high excretion of ammonia and the low excretion of sodium chloride are two of the most characteristic features of the urine during fever. The diminution of the carbon dioxide in the blood

is apparently as constant an accompaniment of fever. It would be of some interest, then, to see in how far these three changes run parallel to one another. This is especially so since Hamburger[16] has attempted to explain the chloride retention on the basis of a febrile acidosis.

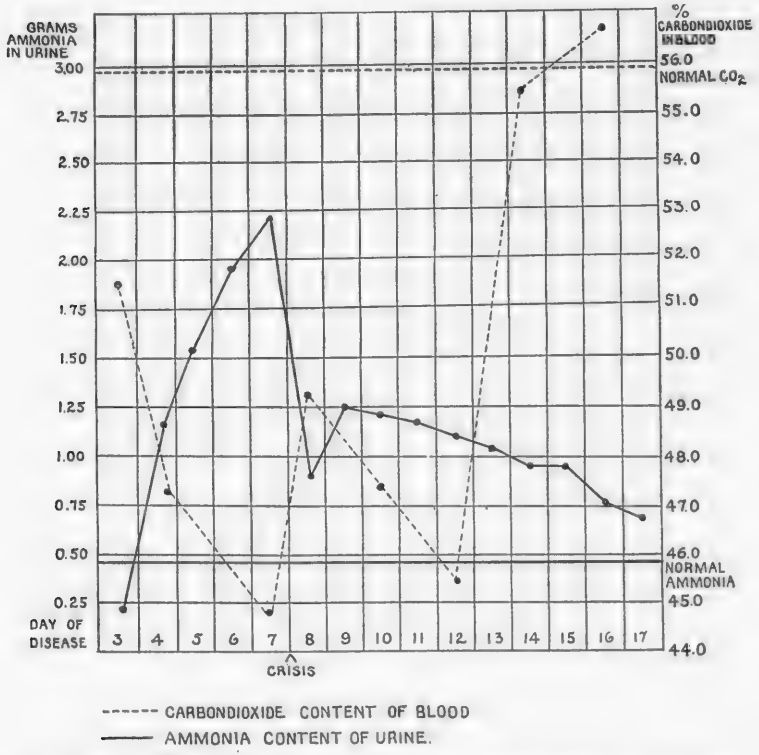
In general, of course, the excretion of ammonia and the excretion of chlorides are in inverse ratio during fever. When the ammonia excretion is high, the chlorine excretion is low. After the fever is over, the ammonia excretion falls to normal and the chlorine excretion rises to normal or above. The curves of excretion of the two substances, however, do not run strictly parallel to one another. The ammonia runs fairly well parallel to the total nitrogen excretion,—so much so that the percentage of ammonia nitrogen is usually not far from normal. Occasionally it is somewhat high during the febrile period. That the changes in chloride and in ammonia excretion do not always run strictly parallel is shown by cases III and VIII (table I), in which the chlorides began to be excreted two days after the crisis, and the ammonia only reached a normal level four or five days later. The same tendency for the ammonia metabolism to readjust itself more slowly than the chloride metabolism is seen, but less strikingly, in other cases.

Kraus[17] has already shown that the carbon dioxide content of the venous blood does not necessarily rise to normal immediately after the fall of temperature. His examinations were made after one or two days of apyrexia. Moreover, we have already seen that in this series of cases the carbon dioxide may persist at a low level for a considerably longer period after the fever has gone. In case III (text-figure 1) it was as low as 45 per cent. five days after the crisis. Two days later it had risen to a normal value. The return of the carbon dioxide to normal was in this instance at just about the period at which the ammonia excretion in the urine was becoming normal. Retention of chlorine had ceased some days before. Case IV shows similarly a lack of relation between the carbon dioxide content and the chlorine excretion. The carbon dioxide was 47.1

[16] Hamburger, H. J., Osmotischer Druck und Ionenlehre in ihrer Bedeutung für die Physiologie und die Pathologie des Blutes, Berlin, 1912.

[17] Kraus, F., *loc. cit.*

per cent. on the eighth day after the crisis, nearly a week after chlorine retention had ceased. The ammonia excretion was irregular but only became constantly normal after this time. In case VIII the carbon dioxide was still low at the time when the chlorine was just beginning to come out.



TEXT-FIG. 1. The relation between the carbon dioxide content of the blood and the ammonia content of the urine in case III.

It is thus only in the few cases in which there is a very definite difference in the time at which the metabolism of ammonia and that of chlorine readjust themselves, that the relation of the carbon dioxide to each of them can be studied. The evidence, however, goes to show that the carbon dioxide in the blood follows much more

closely the curve of ammonia excretion than it does that of chlorine. In view of the known relation between ammonia excretion and carbon dioxide in typical acidosis (experimental acidosis, diabetic acidosis), this is, of course, what one would have expected *a priori*. Hopkins and Davis[18] have brought out the interrelation of carbon dioxide and ammonia from a different point of view by showing experimentally that asphyxiation, by increasing the carbon dioxide content, causes a decrease in the ammonia content of the blood. Case XVII (table II) shows this relationship even more definitely. At a time when the carbon dioxide was 43.64 per cent. in the blood, the patient was given sodium bicarbonate in such amounts that the urine was made alkaline. In the forty-eight hours preceding his death, six specimens of urine were obtained by catheter. Examinations of the blood were made to correspond to each specimen of urine. It will be seen that the administration of alkali caused an increase in the carbonates of the blood (the carbon dioxide pressure was higher than could be read on the manometers), a change in the reaction of the urine, and a great fall in the ammonia excretion. When the sodium bicarbonate was discontinued, the carbon dioxide in the blood fell and the ammonia in the urine rose nearly to its former value. The chlorine excretion showed no rise with the administration of alkali, but rather a gradual falling off until death. The acetone reaction was very strong in all specimens and showed no relation to the carbon dioxide in the blood, the ammonia or reaction of the urine. In the alkaline specimen of urine the amount of albumen was much less than in the acid specimens.[19]

Case XXVI (table III, text-figure 2) shows the same condition. On the third day of the disease, control observations were made on the blood and urine. The carbon dioxide in the blood was moderately low, and the urinary ammonia only slightly high, as is typical of the first days of the infection. The patient was receiving ten grams of sodium chloride by mouth daily in addition to the salt in his food. Of this he was retaining about two-thirds. For the following two and a half days, sodium bicarbonate was given by

[18] Hopkins, R., and Davis, W., *Jour. Biol. Chem.*, 1911, x, 407.
[19] von Hösslin, R., *Deutsch. Arch. f. klin. Med.*, 1912, cv, 147.

TABLE I.

Number of case.	Day of disease.	Maximum and minimum. Temperature.	Pulse.	Respiration.	Percentage of carbon dioxide in the blood.	Total amount of urine in c.c.	Nitrogen in gm.	Ammonia nitrogen. In gm.	Ammonia nitrogen. Percentage of total nitrogen.	Chlorine.	Phosphorus (P_2O_5).	Total acidity of $\frac{N}{10}$ NaOH in c.c.	P_2O_5 acidity of $\frac{N}{10}$ NaOH in c.c.	Free organic acids of $\frac{N}{10}$ NaOH in c.c.	Retained bases of $\frac{N}{10}$ NaOH in c.c.	Ammonia of $\frac{N}{10}$ solution in c.c.	Alkali retention.	Acetone.	Remarks.
I	6	—	—	—	42.83	—	—	—	—	—	—				—	—	—		
	7	—	—	—	34.26	—	—	—	—	—	—				—	—	—		
	8	104.8 102.4	130 124	44 36	47.85	2,220	29.96	2.42	8.07	1.28	3.03				555.0	1605.8	2160.8		
	9	104.0 100.0	128 84	32 24	49.20	1,260	18.72	1.36	7.26	1.18	1.64				226.8	967.9	1194.7		
	10	101.8 99.6	114 76	30 24	—	1,375	21.31	1.08	5.06	0.83	0.48				123.8	764.6	888.4		
	11	101.2 100.6	104 100	32 30	—	1,650	24.97	1.93	7.72	1.00	2.52				363.0	1352.8	1715.8		
	12	102.0 100.0	108 98	32 29	56.27	900	14.13	0.94	6.65	0.55	1.43				220.5	705.8	926.3		
	13	100.8 100.0	100 90	36 29	—	1,380	23.05	1.31	5.68	0.99	3.62				618.5	941.1	1559.6		
	14	101.2 100.6	124 100	36 28	—	1,200	18.70	0.83	4.43	1.75	3.12				498.0	588.2	1086.2		
	15	102.0 100.4	104 104	32 28	—	925	11.95	0.48	4.01	3.81	2.04				342.3	341.2	683.5		
	16	100.6 99.4	96 90	26 26	—	1,150	17.85	0.95	5.32	3.76	3.55				598.0	617.6	1215.6		
	17	100.4 99.6	92 86	28 26	—	1,000	14.72	0.74	5.02	4.73	3.39				535.0	529.4	1064.4		
	18	100.2 99.0	92 86	24 24	57.83	1,000	10.84	0.49	4.52	6.69	2.32				405.0	352.9	757.9		
	19	99.8 98.6	96 72	24 24	—	1,400	12.42	0.17	1.36	—	—				—	—	—		
II	5	104.1 103.9	136 112	38 32	51.30	1,390	25.77	2.04	7.92	1.12	3.10	666.8	436.6	230.2	515.1	1447.0	1962.1	++	
	6	104.0 101.3	128 114	38 32	59.80 62.12	1,120	19.40	1.77	9.12	1.25	1.95	376.3	274.6	101.7	299.0	1258.7	1557.7	++	
	7	101.9 100.8	124 102	36 36	62.07	1,510	15.95	1.07	6.72	2.67	1.55	131.1	218.3	—	128.3	758.9	887.2	++	
	8	100.2 99.3	102 88	36 26	58.50	1,710	14.26	0.72	5.06	2.92	1.98	164.2	278.8	—	111.1	510.7	621.8	++	
	9	99.6 99.0	102 80	32 24	55.11	1,225	13.58	0.60	4.31	3.30	2.27	161.7	319.7	—	131.1	427.0	558.1	+	
	10	99.7 99.0	96 68	32 24	—	1,830	10.40	0.61	5.89	4.05	1.92	195.4	270.4	—	143.5	435.3	578.8	+	
	11	99.8 98.3	92 72	28 24	—	1,320	12.27	0.79	6.45	4.49	2.46	287.8	346.4	—	215.2	564.7	779.9	+	
	12	99.6 98.3	88 72	26 24	—	1,980	15.12	1.19	7.86	6.43	2.66	170.3	374.6	—	188.1	847.0	1035.1	+	
	13	99.9 97.6	92 72	32 24	—	1,345	11.04	1.05	9.58	5.36	2.42	Alk.	340.8	—	Alk.	—	—		
	14	98.6 97.6	92 72	24 20	51.65	1,710	11.71	0.65	5.54	5.47	2.87	496.0	404.2	91.8	384.7	464.7	849.3		
	15	98.4 97.0	92 76	22 20	—	1,940	13.19	0.85	6.14	8.12	3.43	500.6	483.0	17.6	426.8	—	—		
	16	98.0 96.8	84 70	24 20	—	2,270	15.28	1.07	7.04	16.67	3.28	163.5	461.9	—	374.5	761.5	1136.0		

	13	97.6	72	24	—	1,345	11.04	1.05	9.58	5.36	2.42	Alk.	340.8	—	Alk.	—	—	
	14	98.6 97.6	92 72	24 20	51.65	1,710	11.71	0.65	5.54	5.47	2.87	496.0	404.2	91.8	384.7	464.7	849.3	
	15	98.4 97.0	92 76	22 20	—	1,940	13.19	0.85	6.14	8.12	3.43	500.6	483.0	17.6	426.8	—	—	
	16	98.0 96.8	84 70	24 20	—	2,270	15.28	1.07	7.04	16.67	3.28	163.5	461.9	—	374.5	761.5	1136.0	
	17	98.8 97.6	96 80	24 22	—	1,635	12.33	0.86	7.00	7.17	2.96	91.5	416.9	—	130.4	612.0	742.4	
	18	98.4 98.2	106 80	22 18	53.33	1,435	11.70	0.76	6.47	7.34	2.80	490.8	394.3	96.5	361.6	541.1	902.7	
	19	98.3 98.0	112 76	20 18	—	1,320	9.93	0.68	6.84	5.04	2.73	425.1	384.5	40.6	304.9	482.3	787.2	
	20	98.2 97.1	112 76	24 24	—	1,710	10.74	0.81	7.52	9.64	2.98	478.8	419.7	59.1	311.3	567.4	887.7	
	21	98.0 97.0	96 72	22 18	—	1,720	12.77	0.78	6.08	8.34	3.10	595.1	436.6	58.5	375.0	552.9	927.9	
	22	98.6 97.8	96 68	20 18	—	1,520	10.18	0.71	6.99	8.82	2.65	477.3	373.8	103.5	425.6	505.3	930.9	
	23	98.4 97.6	108 72	22 18	53.95	1,050	8.63	0.62	7.15	7.56	2.34	455.8	329.5	126.3	532.0	441.2	964.2	
III	2	104.4 104.2	112 108	30 28	51.31	—	—	—	—	—	—	—	—	—	—	—	—	Received 10 gm. of sodium chloride daily.
	3	104.4 103.6	112 100	36 32	47.30	1,340	25.01	0.23	0.92	0.62	4.22	452.0	594.3	—	274.8	1647.0	439.5	
	4	104.2 103.8	112 108	32 28	—	1,290	26.97	1.17	4.34	1.56	2.97	740.6	415.4	325.2	590.9	735.2	1326.1	
	5	104.2 101.8	112 104	36 36	—	1,335	24.91	1.56	6.30	3.28	2.17	559.7	305.6	254.1	463.3	1005.7	1469.0	
	6	104.0 102.4	112 100	36 36	—	1,580	29.00	1.99	6.86	4.29	2.21	575.1	311.2	263.9	505.6	1411.7	1917.3	
	7	103.0 98.4	106 80	40 28	44.97	1,330	28.19	2.22	7.89	3.08	1.95	481.5	274.6	206.9	401.7	1588.1	1989.8	
	8	98.6 97.4	80 76	24 22	49.26	1,110	24.03	0.93	3.86	2.92	1.94	353.0	273.2	79.8	264.2	664.6	928.8	
	9	98.2 97.4	80 72	28 28	—	1,515	21.60	1.25	5.81	7.29	2.62	287.8	369.0	—	204.5	882.3	1086.8	
	10	98.4 97.4	74 72	24 22	47.23	2.295	19.40	1.21	6.24	15.86	3.42	291.1	481.6	—	149.1	858.7	1007.8	
	11	98.8 98.2	68 56	22 20	—	3.480	16.98	1.15	6.80	17.71	3.21	348.0	452.1	—	330.6	823.4	1154.0	
	12	99.4 98.3	72 60	22 20	45.23	2,815	15.35	1.09	7.12	12.48	2.93	490.9	412.6	78.3	422.3	764.6	1186.9	
	13	99.2 98.1	88 84	22 20	—	3.470	17.53	1.03	5.89	18.59	3.19	562.2	449.2	113.0	416.4	735.2	1151.6	
	14	99.2 97.8	88 76	20 20	55.22	3.460	17.28	0.97	5.74	14.85	3.39	556.4	477.4	79.0	453.3	678.1	1131.4	
	15	99.6 98.0	84 72	20 20	—	4.360	17.61	0.94	5.35	21.99	4.34	640.1	611.2	28.9	553.8	670.5	1224.3	
	16	99.0 98.0	88 72	20 20	56.89	3.200	13.73	0.76	8.70	18.61	3.10	394.3	436.6	—	374.4	541.1	915.5	
	17	99.1 98.2	88 72	24 20	—	2,950	15.16	0.73	4.83	20.31	3.72	511.0	523.9	—	451.3	517.6	968.9	

IV	7	—	—	—	47.78	—	—	—	—	—	—				—	—	—	
	8	102.3 99.8	114 80	52 36	—	1,610	25.08	1.03	3.9	1.17	0.83				249.5	735.3	984.8	
	9	99.4 98.6	84 80	36 28	44.32	1,670	23.62	1.03	4.3	3.95	1.57				250.5	735.3	985.8	
	10	99.4 98.4	88 78	32 28	—	—	—	—	—	—	—				—	—	—	
	11	98.8 96.8	84 72	32 24	—	1.610	22.29	0.59	2.6	4.49	4.39				395.2	417.6	812.8	
	12	99.0 97.8	84 74	26 22	44.58	1,300	17.38	0.50	2.8	5.04	3.29				526.5	355.8	882.3	
	13	97.8 96.4	80 68	28 24	—	1,600	21.15	0.83	3.9	7.71	3.66				646.0	588.2	1234.2	
	14	88.0 97.2	78 64	22 20	—	1,740	25.09	1.23	4.9	6.96	5.06				887.4	876.4	1763.8	
	15	98.0 97.8	80 76	24 24	—	810	12.50	0.49	3.9	3.34	2.11				421.2	347.0	768.2	
	16	97.8 97.6	68 68	24 18	47.10	2,000	24.92	1.10	4.4	11.64	3.72				740.0	782.3	1522.3	
	17	98.6 98.0	80 74	24 20	—	1,120	14.07	0.54	3.8	5.98	1.74				408.8	382.3	791.1	
	18	98.2 97.8	80 68	24 20	—	1,920	21.20	0.93	4.3	15.59	2.43				479.0	664.6	1143.6	
	19	98.2 97.6	72 58	24 20	—	1,575	18.49	0.84	4.5	11.55	2.31				511.8	600.0	1111.8	
	20	98.0 97.6	84 68	24 22	—	1,150	13.59	0.55	4.0	9.27	1.30				296.0	388.2	684.2	
	21	98.2 97.0	80 72	24 24	58.27	1,900	16.32	0.67	4.1	12.21	1.50				256.5	476.4	732.9	
	22	98.2 98.0	92 68	24 24	—	2,500	15.83	0.70	4.4	13.09	1.66				575.0	500.0	1075.0	
V	6	102.4 99.4	100 80	28 24	51.75	1,940	19.39	1.39	7.16	0.52	1.92	384.1	270.4	113.7	291.0	990.6	1281.6	
	7	102.0 100.0	86 68	22 16	54.27	1,720	17.63	1.49	8.43	0.46	2.46	443.8	346.4	97.4	344.0	1060.8	1404.8	
	8	100.3 99.0	80 68	24 18	—	2,360	17.58	1.18	6.71	0.69	2.48	533.3	349.2	184.1	318.5	840.1	1158.6	
	9	99.6 98.8	78 72	20 18	59.17	1,945	14.10	1.00	7.12	0.99	2.57	494.1	361.9	132.2	243.1	712.0	955.1	
	10	99.6 98.6	78 64	20 16	—	2,120	12.84	0.87	6.77	3.85	2.33	510.0	328.1	181.9	296.8	619.4	916.2	
	11	99.4 97.6	72 52	24 18	—	1,965	12.89	0.78	6.04	6.22	2.23	239.7	314.0	—	179.5	555.3	734.8	
	12	98.4 97.8	70 60	18 16	—	2,100	15.39	0.85	5.51	7.43	2.63	508.3	370.4	137.9	262.5	605.2	867.7	
	13	98.4 97.4	72 60	18 16	56.94	2,490	14.74	0.73	4.93	9.59	2.57	562.7	361.9	200.8	174.3	519.7	694.0	
	14	98.4 97.0	72 56	18 16	—	2,185	15.52	0.89	5.72	5.08	3.06	688.9	430.9	258.0	371.5	633.6	1005.1	
	15	97.8 97.2	76 72	20 18	60.21	2,345	15.15	0.88	5.78	11.00	2.25	555.2	316.9	238.3	398.6	626.5	1025.1	
	16	98.6 97.0	76 60	18 16	—	1,820	14.67	0.87	5.91	23.38	2.71	509.7	381.6	128.1	458.6	619.4	1078.0	

	14	98.4 97.0	72 56	18 16	—	2,185	15.52	0.89	5.72	8.08	3.06	688.9	430.9	258.0	371.5	633.6	1005.1		
	15	97.8 97.2	76 72	20 18	60.21	2,345	15.15	0.88	5.78	11.00	2.25	555.2	316.9	238.3	398.6	626.5	1025.1		
	16	98.6 97.0	76 60	18 16	—	1,820	14.67	0.87	5.91	23.38	2.71	509.7	381.6	128.1	458.6	619.4	1078.0		
VI	4	105.4 103.0	132 100	42 28	50.23	1,465	23.82 23.03	1.14	4.78	0.50	2.04	470.1	287.3	182.8	413.1	801.7	1214.8		
	5	102.4 99.6	104 80	28 26	50.12	1,730	29.22 28.73	1.76	6.00	0.88	2.31	491.4	325.3	166.1	449.8	1252.8	1702.6	—	
	6	105.0 98.6	104 58	28 22	48.82	2,170	22.94	1.39	6.07	0.83	0.85	316.9	119.7	197.2	260.4	994.0	1254.4	—	
	7	99.4 98.2	72 54	24 22	—	1,500	23.09	1.68	7.57	0.73	2.48	—	349.3	—	397.5	1194.0	1591.5	—	
	8	99.6 98.8	60 48	24 20	55.64	1,885	21.82	1.29	5.89	0.72	2.28	501.6	321.1	180.5	347.8	917.6	1265.4	—	
	9	98.8 97.5	60 48	24 22	—	1,810	19.18	1.06	5.54	1.45	3.51	579.3	494.3	85.0	398.2	764.6	1162.8	—	
	10	98.0 97.3	72 48	22 18	—	2,045	16.81	0.69	4.13	4.49	3.64	658.6	512.7	145.9	419.3	488.2	907.5	—	
	11	98.2 97.8	54 48	20 18	—	1,635	17.03	0.78	4.58	5.93	4.09	697.9	576.0	121.9	518.3	552.9	1071.2	—	
	12	98.0 97.4	70 52	20 18	60.68	1,775	15.58	0.71	4.56	10.05	3.56	598.5	501.4	97.1	458.0	505.8	963.8	—	
	13	97.6 97.4	72 59	18 18	—	1,920	14.48	0.76	5.25	9.33	3.42	596.4	481.6	114.8	444.4	541.1	985.5	—	
	14	98.2 97.6	72 48	18 16	—	1,915	14.13	0.63	4.48	10.49	2.90	566.7	408.4	158.3	477.5	448.4	925.9	—	
	15	98.0 97.0	60 48	18 16	59.17	1,650	15.67	0.71	4.55	12.44	3.25	656.7	457.7	199.0	495.0	505.3	1000.3	—	
	16	98.2 97.6	72 58	18 18	—	2,100	14.03	0.66	4.70	8.90	2.12	621.8	298.5	323.3	399.0	469.7	868.7	—	
	17	98.0 97.0	88 64	18 16	—	1,540	13.99	0.58	4.14	12.21	3.14	434.3	442.2	—	454.3	412.8	867.1	—	
	18	98.0 97.8	74 60	20 18	57.51	1,650	13.99	0.57	4.06	20.30	3.38	640.3	476.0	164.3	462.0	405.7	867.7	—	
VII	3	101.5 99.2	96 76	36 26	52.31	800	22.16	0.76	3.45	1.125	2.113	610.6	297.1	313.5	384.0	540.8	924.8	++	Received 10 gm. of sodium chloride daily.
	4	100.0 98.6	68 64	28 26	—	1,120	22.63	0.86	3.78	2.54	1.66	596.0	233.8	362.2	328.2	612.0	940.2	+	
	5	99.4 98.6	68 60	24 24	—	1,980	16.69	0.57	3.44	9.74	3.55	1215.0	500.0	715.0	481.1	405.6	886.7	+	
	6	99.2 98.2	56 48	22 20	—	2,590	23.81	0.56	2.37	16.63	5.00	885.9	704.2	181.7	764.0	398.5	1163.5	+	
	7	98.7 97.4	58 52	24 22	—	1,310	14.46	0.46	3.15	9.53	3.55	481.0	500.0	—	411.3	327.3	738.6	+	
	8	98.6 97.8	60 56	28 22	—	1,640	17.04	0.60	3.51	12.40	3.75	506.4	528.2	—	426.4	427.0	853.4	Very slight	
	9	98.8 97.4	64 50	24 20	—	1,850	18.43	0.78	3.30	14.85	3.96	544.2	557.7	—	471.8	555.1	1026.9	0	
	10	99.4 98.2	80 64	20 20	—	1,365	13.32	0.69	5.18	9.98	2.98	527.0	419.9	107.1	473.6	490.0	963.6	0	
	11	99.0 97.6	84 52	24 20	54.37	1,340	12.89	0.62	4.82	12.88	3.15	536.0	443.6	92.4	450.0	441.2	891.2	0	

VIII	4	104.8 104.0	122 108	36 24	47.80	1,060	16.18 15.14	0.77	5.12	0.09	1.73	341.4	243.6	97.8	304.2	547.0	851.2	
	5	104.5 103.8	118 102	32 28	46.53	1,060	16.28	0.91	5.60	0.13	1.55	366.7	218.3	148.4	298.8	647.0	945.8	—
	6	104.8 103.2	116 92	36 28	45.29	—	—	—	—	—	—	—	—	—	—	—	—	—
	7	104.0 102.9	108 92	40 24	48.82	1,870	20.53	0.97	4.74	0.24	1.70	362.7	239.4	123.3	205.7	688.2	893.9	—
	8	103.4 100.3	106 80	36 28	—	3,030	23.12	1.53	6.64	0.37	2.22	660.6	312.6	348.0	399.9	1088.1	1488.0	—
	9	101.5 98.6	96 72	30 24	—	2,800	21.09	1.44	6.82	0.34	2.49	669.1	350.7	318.4	411.6	1023.5	1435.1	—
	10	99.0 98.3	78 60	24 22	—	1,850	—	—	—	—	—	666.1	—	—	421.8	—	—	—
	11	99.0 98.2	66 54	24 22	47.39	1,395	17.64	0.77	4.36	1.13	3.42	605.6	481.6	124.0	481.2	547.0	1028.2	—
	12	98.4 97.4	60 58	20 18	—	1,742	17.92	1.15	6.46	8.51	3.53	748.9	497.1	251.8	494.6	764.6	1259.2	—
	13	98.0 97.0	72 54	22 16	—	3,160	17.06	1.15	6.74	14.03	3.07	689.0	432.3	256.7	521.4	764.6	1286.0	—
	14	98.4 97.1	68 52	18 18	—	1,685	15.92	1.00	6.31	8.58	3.74	778.5	526.7	251.8	615.1	711.7	1326.8	—
	15	98.0 97.0	72 52	20 18	—	2,020	15.24	0.90	5.89	15.94	3.37	585.9	474.6	111.3	575.7	640.5	1216.2	—
	16	98.0 97.8	72 64	20 16	—	1,530	16.85	0.92	5.49	11.78	3.81	847.8	536.6	311.2	673.3	654.8	1328.1	—
	17	98.0 96.8	72 60	20 18	—	1,650	14.95	0.86	5.78	13.38	3.74	848.4	526.7	321.7	622.0	612.0	1234.0	—
	18	98.3 97.0	78 60	20 16	55.17	1,720	17.01	0.96	5.66	15.16	3.70	867.0	521.1	345.9	663.9	683.0	1346.9	—
IX	2	105.0 105.0	120 104	48 40	59.33	—	—	—	—	—	—	—	—	—	—	—	—	—
	3	104.8 103.6	120 116	48 44	53.82	1,320	16.09	1.52	9.20	1.30	2.67	624.7	376.0	248.7	508.3	1101.7	1610.0	
X	3	—	—	—	57.10	—	—	—	—	—	—	—	—	—	—	—	—	—
	4	104.4 103.5	112 100	28 22	48.93	1,250	15.50	0.75	4.87	3.35	1.89	355.0	266.1	88.9	231.3	533.7	765.0	—
	5	104.3 102.9	116 104	34 26	49.94	1,335	15.53	0.99	6.43	5.05	—	186.9	—	—	146.9	704.5	851.4	—
XI	4	102.6 103.8	114 120	28 28	52.44	1,275	23.21	1.30	5.60	2.31	4.20	188.0	591.5	1288.5	771.4	923.5	1694.8	
	5	103.8 102.8	144 128	40 32	—	855	17.57	1.11	6.30	2.80	1.20	557.6	169.0	388.6	294.9	789.9	1084.8	
XII	6	106.2 105.6	140 134	60 56	49.08	1,160	21.92	1.49	6.70	0.0	2.27	719.3	319.7	399.6	475.6	1060.4	1536.0	
	7	—	—	—	46.57	—	—	—	—	—	—	—	—	—	—	—	—	—
XIII	3	103.8 103.0	152 128	52 50	39.65	1,470	21.12	0.69	3.25	1.6	3.68	837.9	518.3	319.6	—	—	—	—
XIV	6	106.5 105.3	112 110	36 32	39.02	—	—	—	—	—	—	—	—	—	—	—	—	—
	7	106.0 105.2	124 108	46 40	39.10	1,430	23.34	1.66	7.12	0.86	3.65	942.6	514.0	428.6	572.0	1181.4	1753.4	
	8	105.4 105.2	130 124	48 44	—	1,190	9.65	2.11	21.87	3.50	2.40	455.5	338.0	127.5	238.0	1501.6	1739.6	

Received 10 gm. of sodium chloride daily.

Received 5 gm. of sodium chloride.

Received 10 gm. of sodium chloride.

TABLE II.

Case 17.

Number of specimen.	Time.	Hours.	Amount in c.c.	Reaction.	Specific gravity.	Albumen.	Nitrogen.	Ammonia.	Percentage of ammonia.	Chlorine.	Percentage of chlorine.	Acetone.	Time.	Carbon dioxide.
I	9:00 A. M. to 6:00 P. M.	9.0	600	Acid	1,021	++	10.36	0.280	2.7	0.42	0.070	++	10:00 A. M.	43.64
II	6:00 P. M. to 5:00 A. M.	11.0	770	Alkaline	1,020	++	7.67	0.033	0.4	0.14	0.018	++	4:30 A. M.	74 +
III	5:00 A. M. to 10:30 A. M.	5.5	640	Alkaline	1,015	++	4.97	0.005	0.1	0.16	0.025	++	9:30 A. M.	74 +
IV	10:30 A. M. to 4:30 P. M.	6.0	490	Alkaline	1,017	++	4.65	0.022	0.4	0.12	0.025	++	2:30 P. M.	62.95
V	4:30 P. M. to 10:00 P. M.	5.5	340	Slightly acid	1,016	++	4.18	0.084	2.0	0.06	0.018	++	8:45 P. M.	55.27
VI	10:00 P. M. to 9:00 A. M.	11.0	355	Acid	1,017	++	4.57	0.271	6.1	0.00	0.000	++	10:00 A. M. 1:00 P. M.	64.62 62.10

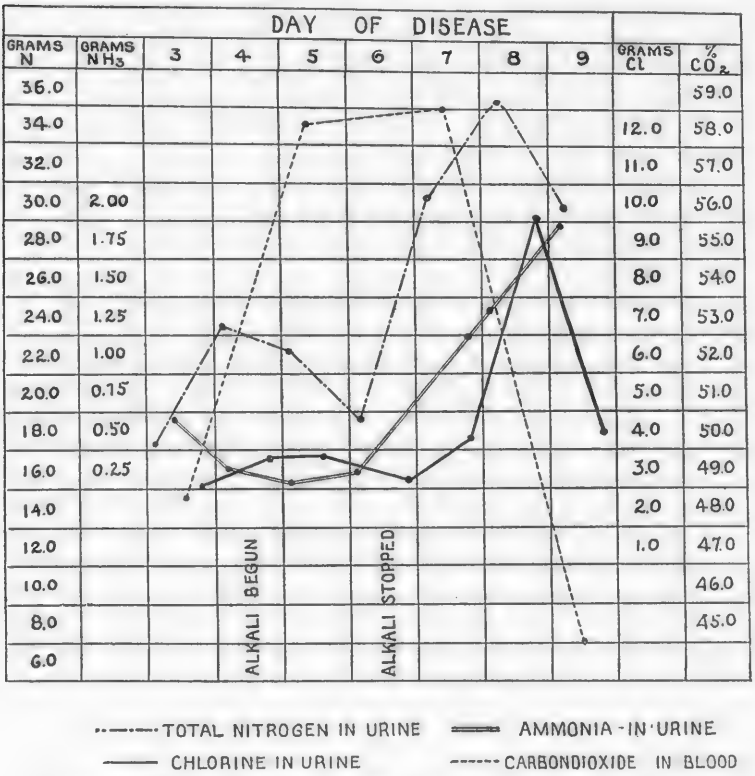
TABLE III.

Case 26.

Day of disease.	Total amount.	Reaction.	Nitrogen.	Ammonia.	Percentage of ammonia.	Chlorine.	Albumen.	Carbon dioxide.	Remarks.
3	1,320	Acid	18.04	0.70	3.8	3.19	Very slight	48.92	
4	2,390	Alkaline	24.65	0.37	1.5	3.74	Slight	—	Sodium bicarbonate 5 gm. every two hours, 9:00 A. M. Sodium bicarbonate 5 gm. every four hours, 8:30 P. M.
5	2,555	Alkaline	22.92	0.34	1.4	3.93	Slight	58.51	
6	2,250	Alkaline	19.79	0.42	2.1	3.33	Very slight	—	Sodium bicarbonate discontinued, 11:30 P. M.
7	2,580	Alkaline	30.70	1.29	4.2	4.38	Very slight	59.15	
8	3,740	Neutral	36.50	1.45	3.9	10.20	Very slight	—	
9	2,320	Acid	30.07	1.96	6.5	4.64	Very slight	45.00	

The patient received 10 gm. of sodium chloride daily.

mouth in rather large doses. The urine became alkaline, the carbon dioxide in the blood rose, the ammonia fell to a low absolute, and a very low relative figure. In spite of a greatly increased diuresis, there was very little change in the chlorine excretion except



TEXT-FIG. 2. The effect of feeding sodium bicarbonate upon the carbon dioxide content of the blood and the ammonia content of the urine in case XXVI.

on the eighth day, after the administration of sodium bicarbonate had been stopped, and when the urine had become neutral once more. On this day, with much the largest total amount of urine, the chlorine rose to over ten grams. The ninth day served as a further

control. The urine was again acid, the ammonia was absolutely very high and relatively slightly high, the chlorine excretion was a little higher than before and the carbon dioxide content of the blood was once more low. In none of the specimens was there more than a faint cloud of albumen. Thus the question of chloride retention was not complicated by acute nephritis.

In these two cases, then, with great variations in the carbon dioxide content of the blood, exactly corresponding changes were found in the ammonia output in the urine. No similar interrelation was to be found between the carbon dioxide and the excretion of chlorine.

So far, then, the evidence points against the theory that the retention of sodium chloride in fever depends on the acidosis. Further evidence against such a theory is given by the conditions found in more typical varieties of acidosis. In diabetic acidosis and in experimental poisoning by mineral acids, there is not a retention of bases as there is in pneumonia, but a marked increase in the excretion of bases. The conditions, then, with regard to the excretion of bases are diametrically opposed to one another.

THE RELATION OF THE CARBON DIOXIDE CONTENT OF THE BLOOD TO THE PHOSPHATES, THE "TOTAL ACIDITY," AND TO THE "ALKALI RETENTION" OF THE URINE.

Phosphates.—In agreement with the usual results in fever, the excretion of phosphates was rather irregular. In general, however, the urine contained larger amounts after the temperature had fallen than in the febrile period. There is apparently no especial relation between the carbon dioxide content of the blood and the excretion of phosphates. The latter frequently rises after the crisis, quite irrespective of any change in the carbon dioxide.

"Total Acidity."—This term is applied by Folin[20] to the value which is obtained by titrating the urine with sodium hydrate after the addition of neutral potassium oxalate, phenolphthalein being used as an indicator. It is expressed in cubic centimeters of decinormal sodium hydrate solution. According to Folin,[21] "the phosphates in

[20] Folin, O., *loc. cit.*

[21] Folin, O., *loc. cit.*, p. 66.

clear acid urine are all monobasic, and the acidity of such urines is ordinarily greater than the acidity of all the phosphates, the excess being due to free organic acids." The acidimetric value of the free organic acids is thus obtained by subtracting the acidimetric value of the phosphates from the "total acidity." "If the acidity calculated from the total phosphates is greater than the titrated acidity, then there are practically no free organic acids present, and the titrated acidity represents the amount of phosphates present in the diacid form."[22] Magnus-Alsleben[23] used this method in the examination of the urine of children with scarlet fever, and found a considerable increase in the organic acids during the febrile period. After the temperature became normal, the phosphate acidity usually exceeded the "total acidity." The children were on a milk and vegetable diet. He believes that the method gives a simple means of obtaining a numerical value for the abnormal excretion of acids during fever.

Pick[24] has described a similar condition in the urine of pneumonia. In forty-two out of fifty-four cases there was a change in the reaction of the strongly acid urine, so that in thirty-six to forty-eight hours after the crisis it became less acid, amphoteric, or alkaline to litmus. This was associated with a drop in the proportion of monobasic phosphates as calculated by him. A simultaneous rise in the excretion of dibasic phosphates occurred, and this he considered to be associated with an increased excretion of bases which had presumably been absorbed from the pulmonary exudate. The importance of the rôle of the exudate is, however, somewhat lessened by the fact that the same condition may be found in scarlet fever.

In the present series of cases the results of Magnus-Alsleben are to a considerable extent confirmed. Values representing a rather high excretion of organic acids were practically constant during the febrile period. In several cases (Nos. II, III, and VII) after the fall of temperature, the urine contained no organic acids and the acidimetric value of the phosphates exceeded the "total acidity."

[22] Folin, O., *loc. cit.*, p. 55.
[23] Magnus-Alsleben, E., *Ztschr. f. klin. Med.*, 1911, lxxiii, 428.
[24] Pick, F., *Deutsch. Arch. f. klin. Med.*, 1900, lxviii, 13.

In other cases, however (Nos. V, VI, and VIII), the excretion of organic acids was apparently as great after the fall of temperature as during the fever. No parallelism could be found between either the "total acidity" or the excretion of organic acids, as determined by this method, and the carbon dioxide content of the blood.

"*Alkali Retention.*"—The method of determining "alkali retention" has been discussed above. The result is supposed to give a more accurate index of the degree of acidosis present than does the determination of ammonia alone. In the present cases of pneumonia the values which represent "retained bases," and which were obtained directly by titration of the urine to the reaction of blood, were variable and not particularly suggestive. The results obtained for "alkali retention" were, in general, somewhat higher during the fever than after it, but the variation is chiefly due to the ammonia excretion. The relation of the "alkali retention" to the carbon dioxide content of the blood is less definite than is that of the ammonia alone.

ATYPICAL CASES.

It has been stated that two cases (Nos. II and V) were atypical in that the carbon dioxide content of the blood was normal or above normal during the febrile period. One of these (No. V) was a case of influenzal pneumonia with involvement of the right lower lobe, and the other a case of pneumococcus pneumonia with solidification of the left lower lobe. The first observation on the carbon dioxide of each case was slightly below normal, but all subsequent observations were normal or above normal. Both were fairly severe cases. The urinary analyses gave results which do not differ from those in ordinary cases of pneumonia,—a moderately high ammonia output, and a marked or moderate retention of chlorides. The diet was essentially the same as in the other cases, and contained nothing which would raise the carbonates in the blood. The cause of this unusually high carbon dioxide is suggested by the analyses of the oxygen content of the blood made at the same time. In uncomplicated cases of pneumonia the diminished respiratory surface is compensated for, and the amount of oxygen in the venous blood re-

mains fairly well within the normal limits of 11 to 12 per cent. In no other uncomplicated cases were oxygen values found which were nearly as low as in these two cases. Both cases, moreover, showed clinically a cyanosis which was more extreme than was seen in any of the other cases. Cyanosis, a low content of oxygen, and a high content of carbon dioxide, suggest, of course, an interference with the respiratory gas exchange, an incomplete absorption of oxygen and an incomplete excretion of carbon dioxide. In case II, at the first observation, the oxygen content of the blood was normal and the carbon dioxide was, as one would expect, low. On the following days, when the oxygen was low, the carbon dioxide had risen markedly, and only when the oxygen rose again to normal did the carbon dioxide fall. Practically the same association of low oxygen and high carbon dioxide is seen in case V.

Such an imperfect aeration of the blood might conceivably depend on an incomplete compensation for the diminution of respiratory surface caused by the pneumonic exudate, although it is scarcely possible that the throwing out of one lobe should produce so profound a change. According to Loewy and von Schrötter,[25] the occlusion of the main bronchus to one lung, without change in rate of respiration or circulation, would only lower the oxygen content of the venous blood to 8 per cent. Moreover, in case V the low oxygen and high carbon dioxide persisted for some time after the chest was perfectly clear. That the low oxygen was due to an inability of the blood to take up oxygen normally was disproved in case V at least by finding, on three occasions, that the combining power of the blood for oxygen was normal. That the time of the reaction might be slowed, however, cannot be excluded. In neither case was there any reason to believe that improper aeration depended on too shallow respiration. In case V the pulse rate was not rapid, but in case II it was about what one would expect with the amount of fever present. An unusual slowing of the rate of circulation would increase the amount of oxygen given off in the capillaries, and also the amount of carbon dioxide taken up, and thus account for the changes found in the venous blood. Wolff[26] has

[25] Loewy, H., and von Schrötter, H., *Ztschr. f. exper. Path. u. Therap.*, 1905, i, 197.

[26] Wolff, E., *Arch. f. exper. Path. u. Pharmakol.*, 1885, xix, 265.

shown that in rabbits the circulation in fever is actually slower than normally. More recently Hewlett[27] has concluded from studies on man that "the flow is exceptionally slow when the temperature rises during fever and is moderately accelerated when the temperature falls during fever." If this be so, then in the great majority of cases of pneumonia, the slowing of the circulation, as well as the diminished respiratory surface, must be compensated for by changes in respiration. From the facts at hand, however, it would be impossible to determine whether the unusual condition of the blood in these cases is due to a slowing of the circulation or to an interference with respiratory exchange of gases due, possibly, to some local change in the lungs, as of the capillary walls or the alveolar cells, or to the effect of a change in the chemical constitution of blood cells or of serum. Kraus found that the carbon dioxide content of the blood was considerably higher in pneumonia than in other acute fevers, and he explained this by the pulmonary involvement which complicates the conditions in pneumonia. According to him, the local conditions interfere with the ready excretion of carbon dioxide. In the presence of a normal oxygen content, as is found in most cases, this would be difficult to prove, but the explanation seems worth considering in these atypical cases.

CONCLUSIONS.

A diminution in the carbon dioxide content of the blood is a constant feature in pneumonia. Occasional cases, however, may fail to show low carbon dioxide.

The carbon dioxide in the blood bears little definite relation to the severity of the disease, except that it tends to be lowest in severe cases and in the terminal stages of the disease. There is less deviation from the normal in short or mild cases.

The diminution in the carbon dioxide in the blood bears no immediate relation to temperature, as it may persist for some days after the patient is afebrile. The diminution in carbon dioxide corresponds to the other evidences of metabolic changes in infection and, like them, may be even greater after than during the febrile period.

[27] Hewlett, A. W., *Heart*, 1910–11, ii, 230.

The changes in the carbon dioxide content of the blood run parallel to the output of ammonia in the urine. The carbon dioxide appears to bear no relation to chlorine excretion.

In two unusual cases the carbon dioxide content of the blood was normal or above normal. This was associated with a very low oxygen content of the venous blood.